UNIVERSITY OF TORONTO
3 1761 01570791 2

EXPERIMENTAL PLANT PHYSIOLOGY

BY

GEORGE JAMES PEIRCE
Professor of Botany and Plant Physiology
Stanford University

265157
4 . 3 . 32

NEW YORK
HENRY HOLT AND COMPANY

Copyright, 1931
by
Henry Holt and Company, Inc.

PRINTED IN UNITED STATES OF AMERICA

PREFACE

The following pages reveal an attempt to furnish the evidence on which current conceptions as to the physiology of plants are based. The experiments here described do not involve the skill which should accompany the use of the complicated and delicate apparatus often required by physiological research. On the other hand I have, on principle, made no attempt to substitute lamp chimneys and tomato cans for proper instruments for experiment.

The experiments here described will enlighten the curious as to the qualities and operations of living organisms; they will furnish the evidence from which certain inferences are drawn and on which certain hypotheses or theories are based; but they must not be regarded as furnishing *proof*. We should try as conscious intellectual effort to distinguish between evidence (or indication) and proof, recognizing that much that is called proof is merely evidence in harmony with current conceptions. In this sense these experiments prove little; but they elucidate much.

The experiments in this form have been tried with my elementary students here and found practicable. I have naturally referred them most frequently to my own book for discussion; but I have also kept in the laboratory for their use other books, of which I have a list on a neighboring page. I regret that most persons in this country find reading other languages so difficult that references to French, German, and the even lesser known languages, are mainly futile. But for the pursuit of plant physiology professionally one must be able to read more than one's mother language.

I hope that teachers as well as students will be helped by these fruits of experience.

G. J. P.

Stanford University, California.
May, 1931.

CONTENTS

PAGE

PREFACE iii

INTRODUCTION vii

CHAPTER

I. THE PROPERTIES OF CELLS 1

II. ABSORPTION 34

III. THE MOVEMENT OF WATER 51

IV. THE MANUFACTURE OF FOOD 73

V. THE STORAGE OF FOODS 95

VI. DIGESTION 100

VII. OXIDATIVE METABOLISM 108

VIII. GROWTH 122

IX. IRRITABILITY 132

X. REPRODUCTION 160

INTRODUCTION

When one realizes that the ability of living things to live where they do depends upon their ability to carry on their vital processes in harmony with their circumstances, one is led to enquire what these circumstances are, and by what means plants and animals continue to operate under these conditions. The product of such inquiry has still further stimulated curiosity, so that today we have many partial answers to our general question. The student of geographical distribution, of horticulture, agriculture, and forestry, and of other matters in pure and applied science concerning living things, expresses himself in terms which he assembles under certain headings. One of these is Plant Physiology. If one turn to the text-books in this field one will find statements of current opinion and discussions of divergent views, but scanty description of the means of securing the evidence on which views, opinions, and statements rest. Yet experiment is not necessarily difficult, and hence direct acquaintance with both the methods and the foundations of science is possible.

In the following pages are described experiments which furnish the evidence, if not the proof, on which the present science of plant physiology is built. From these introductory and more or less fundamental experiments one may proceed at will to more exacting studies of the many problems suggested by these experiments, the ideas of which they are the foundation, and the economic or other questions on which light may be thrown by physiological studies. In all our work we should bear constantly in mind the delicate organization of living things, their extreme sensitiveness to their surroundings, the promptness with which they give chemical or other evidence of health, decline, and death or recovery. The physiologist is obliged to work with living things, and to have them as nearly normal in condition and circumstances as possible. His difficulties in this respect are very great; but if he take pains in the first place to acquaint himself as intimately as possible with certain living things he will find himself understanding their behavior better and better and seeing deeper and deeper into unsolved problems. We may begin, therefore, with living cells and living protoplasm.

RECENT BOOKS ON PLANT PHYSIOLOGY

Barton-Wright, E. C. *Recent Advances in Plant Physiology.* Philadelphia, 1930.

Harvey, R. B. *Plant Physiological Chemistry.* New York, 1931.

Kostychew, S. *Chemical Plant Physiology.* Translated by Lyon. Philadelphia, 1931.

Maximow, N. A. *A Textbook of Plant Physiology.* Translated and edited by Murneek and Harvey. New York, 1930.

Miller, E. C. *Plant Physiology.* New York, 1931.

Peirce, G. J. *The Physiology of Plants.* New York, 1926.

Raber, O. *Principles of Plant Physiology.* New York, 1928.

CHAPTER I

THE PROPERTIES OF CELLS

In order to form some idea of the mechanism which performs those operations which collectively constitute living, we should make such examinations of living cells as the tools now at our command permit. These tools are the microscope, chemistry, and physics. To use the microscope to best advantage a technique has been developed which, in certain instances, involves the killing of the living organism. Many of the methods of physics and chemistry are also fatal to the organisms under investigation. It is therefore necessary to observe great caution in drawing conclusions from the results obtained by using such methods; but it is also no less true that, without the application of such "unnatural" methods to the world of Nature, we should know even less than we do now about living things. The advantage of beginning a study of living organisms with observation of them alive is, however, so evident that one need hardly emphasize it. One may even practice it.

Plants have bodies composed of larger or smaller numbers of units called *cells*. These consist of protoplasm and its products. Whereas the bodies of animals consist of cells, all or nearly all of which continue to live as long as the animal does, the bodies of the larger land plants contain many structural units no longer living. We may then, if we choose, distinguish between these physiological units or living cells, that perform the operations which we say constitute living, and those structural units which may or may not continue to live but which, in either case, perform necessary mechanical work. Thus the wood of many trees, and their outer bark, are composed of the empty shells (walls) of once living cells. These shells constitute the skeleton and the covering between which the living cells lie. This can be demonstrated by sectioning.

Cells. On making a thin cross section of such a woody stem as that of buckeye (*Aesculus hippocastanum* or *A. californica*)—for example of a branch perhaps a quarter inch in diameter—or of such an herbaceous stem as buttercup (*Ranunculus*), geranium (*Pelargonium*), or chrysanthemum (*Chrysanthemum*) one finds that the surface of the plant is composed of a layer or layers of cells purely protective in present function. The buckeye is covered by lifeless cell walls only, whereas the sections of the herbaceous stems show living epidermal cells on the outside. In the woody part of these stems one sees that the cambium, medullary rays, and parts of the sapwood consist of living cells, and that the fully developed ducts and fibres contain no living protoplasm. In longitudinal sections one may confirm and extend these observations.

On the other hand it is easier to study living cells which do not require to be uncovered in order to be visible. Hairs have long furnished the classical objects for the study of living cells in land plants. The hairs on the growing tips of squash and pumpkin vines (*Cucurbita pepo* and other species) and the stamen hairs of flowering branches of various species of *Tradescantia* (Wandering Jew, spiderwort, etc.) offer advantageous material. Many other plants are hairy, but in too many instances the cells die soon after forming the hair. Examination of even growing tomato vines proves this to be true.

The Structure of Cells. If we examine a young squash or pumpkin hair, or a stamen hair of Tradescantia, we find it to be made of a single row of cells, generally elongated (any exceptions?) placed end to end, and themselves tapering from base to tip. The side walls of these tapering cells are directly in contact with the air, the end walls (any exceptions?), on the other hand, are in contact with other cells. The environment of these cells is therefore different on the sides and on the ends, and the respects in which the two differ should be realized. The air is a mixture of gases, or a solution of gases in a gas, exercising a pressure of about fifteen pounds upon every square inch exposed to it. Any structure or system, therefore, incapable of withstanding at least this pressure, will be crushed. But the walls of cells are parts of a system which affords much greater mechanical strength than atmospheric or barometric pressure alone would require. It is noticeable that elongated plant cells very commonly bulge somewhat in the centre. The similar bulge in the shaft of a Greek column was designed by the architect to counteract an optical or mental illusion; while the bulge in the walls of an elongated plant cell is the result of internal pressure (turgor or turgescence) greater than necessary to withstand the balance of atmospheric pressure not carried by the cell wall itself. This balance will be some fraction of fifteen ($\frac{x}{15}$). Thus, suppose a cell wall to be capable of carrying only a pressure of five pounds to the square inch; then the cell must itself withstand a pressure of ten pounds ($\frac{x}{15}=\frac{10}{15}=\frac{2}{3}$); and if the cell develops more than this pressure, the wall will be correspondingly pushed outward. If the wall is strong and rigid, it will not be stretched; if it is elastic, it will stretch or bulge or even burst. The pressures on opposite sides of an end wall may be similar or quite different in magnitude from those on the side wall. If the pressures on opposite sides of a wall are equal, the wall itself may be very thin and weak and straight. If they are unequal, the wall cannot be any of these.

If, now, one compare the walls of cells composing a hair with the walls of cells composing the wood at the butt of a tree fifty or more feet tall, one will see a great difference in the mechanical structure of the two. The former is relatively thin, the latter very thick. This comparison can readily be made under the microscope by boring out a plug of wood from the butt of a tall tree, sectioning this with a razor, and examining in a 50% glycerine solution in water. Both cross and longitudinal sections will reveal the thickness of the walls.

Reflection and comparison will lead to clear apprehension of this aspect of the mechanics of cells. One realizes that in an automobile tire the mechanically strong casing is greatly strengthened by the pressure against it of the mechanically weak inner tube, which contains compressed air. An intact inner tube is air tight, the casing is not. The pressure in a tire may be regulated according to the load. Just as in a cell, the pressure which must prevail in a tire is equal to the balance found on subtracting the mechanical strength of the casing from the load. For example, if the casing is able to support five pounds per square inch and the total pressure of the car is its weight on the tires, the air pressure in the tires must equal the latter minus the former. Furthermore, there should be a margin of safety, and hence one uses an air pressure in a tire equal to the unusual load, and one finds the pressure in *living* cells to be, not a fraction of one atmosphere, but two or more atmospheres.

Composition of the Cell Wall. Before turning to the question of how this requisite pressure, called *turgor* or *turgescence* or *osmotic pressure,* is developed and maintained (pp. 12-14), the composition of the cell wall should be examined as well as its form and thickness. If one section a piece of elder or sunflower or similar pith and immerse the section in a solution of iodine in water in which potassium iodide is also dissolved (Gram's Iodine I+KI+aq.) and then follow this with strong sulphuric acid, the cell walls will turn blue or black. This is a common microchemical test for cellulose ($C_6H_{10}O_5$), an isomer of starch. But if one similarly apply an iodine solution and sulphuric acid to squash or stamen hairs and to wood sections, the walls do not turn blue or, in the case of the crosswalls in the hairs, only the walls common to adjacent cells turn blue.

The outer or side walls of the cells forming the hairs, and the cells forming the wood, turn yellow. The material of cell walls is, therefore, not all alike. One should now ascertain what these differences imply, for differences in strength, hardness, and relations to water, are very significant.

If one apply an alcoholic solution of Phloroglucin to the sections previously described (pp. 3-4), and follow this with hydrochloric acid, one will see that the very thick walls of the wood elements turn red. The same treatment of the stamen hairs or squash hairs fails to produce any red color. The walls of wood elements are *hardened,* with only partial loss of pliancy and elasticity, by substances (so-called *lignin*) formed by the cells while they were alive, and deposited or infiltrated in the cellulose of the walls. This hardening does not impair the passage of water into and out of the material: it increases the mechanical strength of a tissue without altering its water relations.

On the other hand, if one apply an aqueous solution of anilin chloride to squash or stamen hairs and follow this with dilute hydrochloric acid their side (outer) walls turn yellow, while their end walls remain uncolored. The iodine-sulphuric acid test previously made (p. 4) showed that these end walls are cellulose, unchanged. The anilin chloride-hydrochloric acid test applied to strips of epidermis or to sections of leaves will show that only the water proofed outer walls of the epidermal cells turn yellow, while the side and bottom walls remain colorless. The anilin chloride-hydrochloric acid test applied to wood sections also gives a yellow reaction, but since the phloroglucin test shows no lignin in the walls of the hairs or of ordinary epidermis, one can use the phloroglucin test as diagnostic of lignification, and the anilin chloride test as diagnostic of *cutinization* and water proofing. *Cutin,* so called and certainly not a chemical unit, is infiltrated into the exposed walls of cells, which are thus water proofed.

Other infiltrations or impregnations of their walls by living cells may be seen in the diatoms, and in the epidermal cells of horse tails or scouring rushes (*Equisetum*) and grasses (wheat stalks, etc.), in which the walls are hardened but not toughened by deposits of silica (SiO_2); in *cork,* in which the walls are greatly modified in composition and water proofed; in the hard shells of walnuts, etc. On the other hand, the enormously thickened walls of the cells in the endosperm in the nuts of the ivory palm are not infiltrated at all, but are pure cellulose.

Living Cells: Protoplasm. While further studies of this sort will increase one's knowledge of the walls of plant cells, we may now turn to the living cells themselves. If one examine living cells in a drop of ordinary tap or drinking water under the microscope, one sees that the cell wall, with its sharp outer and inner outlines, is completely lined with a finely granular, translucent, and colorless material. Such cells as those forming the stamen hairs of *Tradescantia,* of the hairs on pumpkin and squash, and of the leaves of the aquatic *Elodea,* contain coloring matters; but one should at once distinguish between the colorless, granular *protoplasm,* and the chlorophyll-containing (green) chromatophores or the blue, purple, or reddish cell sap forming the vacuole in the cell. Many other plant cells contain no coloring matters whatever, and the colorless protoplasm is correspondingly more evident. In the protoplasm there may be movement, the directions, speeds, etc., of which will vary, as we may see (p. 10), with the temperature, light, etc. These change from time to time in Nature, and we may deliberately alter them in experiment.

Protoplasm is described as a structure or system consisting of proteins, carbohydrates, fats and oils (lipoids), salts (electrolytes), and water.[1] The proportion of water is always very high (96%+). The relations of water to the other substances are diverse. This is indicated under the microscope by [2] dark-field illumination, in which the light is made to pass through the object in horizontal plane instead of vertical; [1] by the ultra microscope, in which much greater magnifications are possible than in the usual compound microscope; and by [2] physical-chemical manipulation. The water is the solvent in the cases of the salts, some of the carbohydrates, and some of the proteins, which with it form a solution. The fats and oils and some of the proteins are insoluble and are suspended in it. The lipoids and water form an emulsion, in which the water is continuous, and the fats and oils in discontinuous droplets. The proteins and the water form a system, more or less gelatinous according to species and according to circumstances in the same species. The proteins may be continuous, with the water contained as droplets, a *gel,* or the water may be continuous with the protein particles suspended in it, a *sol.* In either case there results a remarkable plasticity whereby the protoplasm can change its shape, conform to any surface or angle, move itself and its parts, and even accomplish locomotion. It flows with more or less ease, according to the proportion and the condition of the water in it, but not with quite the freedom of water at the same temperature. In other words, protoplasm is viscous, and its viscosity is higher than that of water. The viscosity of protoplasm may be *observed* under the microscope to differ in different cells by comparing the rates of Brownian (dancing) movement of the particles in it. Thus the viscosity of the protoplasm in the living, intact cells of stamen and squash hairs, of *Elodea* leaves, and of *Spirogyra* filaments varies respectively from a considerable stiffness (gel) to almost that of pure water (sol) as in *Spirogyra.*[3]

---

[1] See pages 8-18 in Peirce, G. J. *The Physiology of Plants,* New York, 1926, for a fuller discussion.

[2] Belling, F. *The Use of the Microscope.* New York, 1930.

It is a common observation that protoplasm does not dissolve in water. Although some of the constituents of protoplasm exist in it in solution in water, the relations of the proteins and water as just described throw a certain amount of light upon the fact that protoplasm as such remains intact in water. Protoplasm may take up water and swell, but its hydration capacity is fixed and limited. A lump of sugar may be put into the water in a tea cup, a barrel, or a lake, and its component molecules will disperse or diffuse throughout the volume of water, but if a lump of protoplasm is put into water in the same containers, it will definitely retain its limits. In other words the relations of the molecules or minute masses of proteins and of the molecules of water are such that they remain together. They are in fact held together.

---

[1] *Ibid.* p. 93.

[2] Gage, S. H. *The Microscope.* 13th edition or later. Ithaca, 1920 or later.

[3] Becking, L. G. M. and Bakhuyzen, H. v. d. Sande. "The physical state of protoplasm" *Verhandl. d. koninklyke akad. v. Wetenschappen t. Amsterdam.* XXV. 5, 1928.

The force or forces by which they are held together may be indicated by the length of time and the amount of energy required completely to dry, or to drive off the water from, a mass of protoplasm. It will be found, on experiment, not only that protoplasm loses a great deal of water on heating, but that its shape, etc., depend upon it, and that it takes a great amount of energy in the form of heat, to dry a mass of protoplasm to constant weight. Furthermore, once completely dried, the structure or system which we call protoplasm will not be restored merely by restoring the water. We may, therefore, conceive the protoplasm which we see under the microscope as a translucent granular material lining cell walls, as a somewhat viscous liquid in which particles or masses of water and of proteins are in contact with each other, each particle retaining its identity and individuality, each particle of each substance having shape and size as well as position and mass, and each particle having surface as well as mass. Hence, wherever and whenever these particles touch other particles of the same or other sorts, their surfaces touching other surfaces, they are kept from fusing only by the force or forces on their surfaces and in their masses. Thus water and proteins are held together as a system by certain cohesion forces; and they are held apart, so that they do not dissolve in each other, by the tension of the individual surfaces. If the amounts or proportions of water change in such a structure, the particles of other substances will move closer together or further apart. The pliancy and plasticity of protoplasm may change from time to time, but it holds uniformly together as a system. If the system is broken, it is permanently destroyed; for those forces on the surfaces and in the masses of the component particles are not restored merely by the addition of water to protein. Living protoplasm cannot be made in this way: nor has man ever made it in any way.

Movements of Protoplasm. The movements of and in the protoplasm of living plant cells have been extensively studied and described. The circulation, rotation, of the protoplasm as a whole, and the separate movements—contractions and extensions—of its different parts, are closely related to its viscosity; but obviously one should not confuse condition and cause. Movement is freer, involves less expenditure of energy to do a given amount of work, if the viscosity is low. On the other hand, low viscosity is not always coincident with rapid movement, as Spirogyra shows. In fact one is not always able to see movement in protoplasm, no matter what the viscosity. The one general conclusion to be drawn at once from the foregoing observations and discussion is that the phenomena of life, the evidences of living, are complex in causes and conditions. All one can hope to do at this time is to identify some of the causes and to recognize some of the conditions. Thus, viscosity is one of the conditions for movement in protoplasm, but it is not the only one, as may be shown by the following experiments, and it is by no means the cause.

The movements of protoplasm vary in rate according to the temperature. This may easily be observed by examining the behavior of protoplasm in the cells of *Elodea* leaves or in the hairs of Tradescantia and squash, first at room temperature, to establish the rate of movement, and then gradually raising or lowering the temperature and noting the correlation, the lower the temperature the slower the rate, the higher the temperature the faster the rate, within certain limits. By means of warm stages[1] and thermometers, together with eyepiece micrometers, one will be able to note that the speed of movement rises with the thermometer up to about 36° Centigrade. If the temperature be still further raised the movement decreases in speed, ceasing at or below 50° C. If the temperature be now lowered, the movement will probably be resumed; but it will not have the former rate, it will be slower than before. On the other hand, if the temperature be raised to 75° C., which is the temperature employed to pasteurize milk, movement will not be resumed when the temperature is lowered; and if the temperature goes to 100° C. visible changes occur in the protoplasm which convey to the eye and the mind that the protoplasm is coagulated, it has set or solidified, its viscosity has risen very sharply and is practically lost.

On the other hand, having set up the experiment at room temperature,[2] one can decrease the rate of protoplasmic streaming by lowering the temperature. The movement will either become very sluggish or cease altogether at temperatures near 0° C., but it will be resumed if such a temperature be only briefly maintained, though the rate will not return to the one with which the experiment began. If the low temperature be maintained for any length of time, or be reduced to 0° C. or lower, the movement will cease altogether and cannot be made to start again. (See note).

---

[1] There are many types, all troublesome, some expensive, to be had of the Bausch & Lomb Optical Co., Rochester, N. Y., and of the dealers in laboratory supplies, Arthur H. Thomas Co., Philadelphia, Eimer & Amed, New York, etc.

[2] The temperatures above cited are only approximate and relative, for the temperatures required to produce the effects described will vary with the plants employed, the conditions of the plants, etc. Thus I have repeatedly noted the difference in behavior of cells on plants brought into the laboratory early in the forenoon from the garden where they had been exposed to a temperature of about 10° C. for hours during the night; and cells of the same plants in the early afternoon of a sunny summer or autumn day. Greenhouse and garden plants will behave diversely, as every horticulturist knows, for he may "harden" his greenhouse plants by gradual transfer to garden or field.

We can thus distinguish four critical temperatures in relation to protoplasmic movements in these cells, namely fatal *heat,* 75-100° C., fatal *cold* ±0° C., a temperature which produces *heat rigor,* 50° C., and a temperature which produces *cold rigor,* 0-4° C. We cannot fail to wonder what the physical and chemical effects of these temperatures are to cause these results. We can realize that sufficient heat will coagulate proteins, but why should less heat produce a slowing or ceasing of movement in protoplasm, still less heat an acceleration, less and less heat slower and slower movement until movement finally ceases almost or quite entirely. Discussion of this and related subjects is abundant, but one can find a convenient summary, and starting point for further work, in the book to which frequent reference will be made.[1] The concise statements and the experimental evidence accumulating from the pursuit of the directions given above and in the sequel receive critical examination and amplification.

We can make our ideas of the correlation of temperature and protoplasmic movement more definite and vivid by constructing curves on coordinate paper connecting the points at which we have determined temperature and speed. There will be the further advantage in attempting this in that we shall discover that the rate of movement is not necessarily uniform or constant throughout a single cell. If the movement throughout a single cell be not compensated, one part moving with enough speed to make up for the slowness of another part, the distribution of the protoplasm would be quite irregular instead of so strikingly uniform as one sees it to be. One correctly infers that a cell operates as a unit and is a unit, physiological as well as structural.

This view is strengthened by observing that there is no visible movement of particles from one cell to the next. Whatever openings there may be through the end walls are not large enough to permit the passage of particles of perceptible size. There is movement from cell to cell, and there is continuity of protoplasm; but the cellulose wall, while freely passable to water molecules, is not passable by particles not so reduced in size by solution that they become invisible (p. 34).

---

[1] Peirce, *The Physiology of Plants,* pp. 329 ff.

Mechanics of Cells. So far we have learned that living cells are units of structure and function, as said by the great German botanist Sachs; that they are little masses of protoplasm surrounded by a wall which may vary in composition, hardness, strength, and water relations; that in form and color they and their parts may differ very considerably; but we can find out more about the cell as a self-operating mechanism by studying its mechanical properties and the composition of its component parts (p. 15).

An *Elodea* leaf examined under the microscope on a slide, and in the water in which it grows or, ordinarily, in tap water, will be found to consist of a single layer of cells, green in color because of the chloroplasts in the cytoplasm. The cytoplasm lines the walls closely. What causes the cytoplasm to be in such contact with the cellulose wall? If *Tradescantia* stamen hairs, or the hairs from the young branches of squash or pumpkin, are placed *dry* upon a slide and the length and diameter of some of their component cells be determined, it will be found that both length and diameter of these cells will *increase* when one puts tap or distilled water upon the object on the slide; but that both will *decrease* if one apply a sugar or salt solution of due concentration. Manifestly, therefore, living cells are under internal pressure.[1] The nature and amount of this pressure can be determined, and it will be found to be different in different plants and in different circumstances. Thus we may examine *Elodea, Spirogyra,* and other plants of fresh water, the marine and fresh water Cladophoras, etc. and land plants of various sorts, and we shall find that the pressures in their cells are different.

---

[1] This is called *turgor* and the cells in normal condition are said to be *turgescent* or *turgid.* Flowers, etc., wilt because the turgor of their cells falls from loss of water.

If one replace the tap water in which a *Tradescantia* or squash or pumpkin hair is mounted on a slide under a microscope, by 5% potassium nitrate solution (KNO_3) or 3% common salt ($NaCl$) or 10% table sugar ($C_6H_{12}O_6$), the protoplasm will shortly begin to contract away from the walls, beginning at the line where side and end walls join, and one will see that the cell is a sac or bag formerly filling the cellulose casing, but now shrunken away from it below. By testing KNO_3 solutions of various strengths less than 5% one will presently find one which barely contracts the protoplasmic sac (protoplast) and others which do not contract it at all. There is a concentration, therefore, between the solution of lowest concentration which contracts at all, and the solution of highest concentration which does not contract the protoplast, that is exactly equal to the concentration of the cell. Such a solution is said to be *isotonic* with the cell. Other solutions are *hypotonic* or *hypertonic*, according as their concentrations do not equal or exceed the concentration of the cell.

It has been experimentally determined by physical chemists and others that salt and sugar solutions exert very considerable pressures. These pressures are of two sorts. They may be the pressures due to the bombardment, against the walls of the container, of the molecules of the substances in solution; and they may also be due to the volume of the solution. The former is called osmotic pressure, the latter is turgor pressure or sap pressure. The pressure which keeps cells plump depends upon the volume of water within them; but the maintenance of this volume depends upon the absorption of adequate amounts of water to make good the quantities used or lost, and this absorption depends upon the concentration (osmotic pressure) of the solution within the cell (see p. 40). By reducing the volume of water within the cell, either by evaporation (wilting), or by withdrawing it by dehydrating solutions, one will lessen the turgor pressure, the turgescence, and the volume of the cell. Conversely one can increase these last three things, by increasing the volume of water within the cell, either by checking evaporation, or by adding water. The cell wall, then, is pressed upon by the protoplasm, which is so stretched by its water contents that it lies in perfect contact with the inner surface of the cell wall, so long as the cell is alive and well. Wilting, partial dehydration, partial contraction, and collapse, are all signs of abnormally low pressure. This may cause or accompany death. Permanent wilting is often the first evidence of death; and a cell is regarded as dead when, under the microscope, its dehydration and contraction, called *plasmolysis*, cannot be reversed by adding pure or tap water.

The pressure by which the protoplast is held against the cell wall is not under ordinary conditions considerable; but we know from observation of growing roots, etc. that these soft, mechanically weak, organs can, if properly supported, develop great pressures (p. 44). This system, consisting of a mechanically weak inner sac of protoplasm, and an elastic strong outer sac of cellulose or one of its derivatives, is capable of exerting tremendous pressures. The comparison previously made between a plant cell and an automobile tire is entirely fitting and most significant.

The turgor pressure is exerted, not by the protoplasm as such, but by the water within it. The water may be fairly uniformly distributed throughout a compact mass of protoplasm (as in young cambium cells), or it may in addition be accumulated in volumes of larger or smaller size and number, occupying what appear to be cavities or *vacuoles*. In either case, the water of the cell, or cell sap, is a solution of great diversity of composition, though there are always certain elements and compounds in it, and of concentration characteristic of the species, the age, and the circumstances. Turgor or turgescence in youth gives place to wilting or flaccidity in age, in cells as well as in organs and organisms. This is a matter of common observation, but can be tested under the microscope, if one choose, by determining the concentrations, etc. of the cell sap by means of KNO_3 solutions of different proportions.

The Composition of Protoplasm. The application of the usual solutions of iodine to living cells under the microscope produces certain significant results. The usual solutions of iodine are either tinctures, i.e. alcoholic solutions, or aqueous solutions containing one or more salts. Experience shows that iodine, a volatile dark brown solid in the form of flakes, dissolves only very sparingly in distilled water; but that in an aqueous solution of potassium iodide it dissolves freely. On applying solutions of iodine, therefore, either in experiment under the microscope, or in antisepsis as commonly practiced, one must take the other substances as well as the iodine into account in interpreting the effects of the alcohol as well as of the iodine. If one use Gram's or Lugol's aqueous solution of iodine, one must consider the effects of the water and the salt (KI) as well as of the iodine. Experiment will lead to the desired information.

Proteins. Alcohol is a well known dehydrating agent, and a solvent of many fatty and resinous substances. It mixes freely with water in any quantity, and oxidizes (burns) at a moderately low temperature, liberating a considerable amount of heat. If one apply Alcohol Absolute (100%) to living cells under the microscope, the protoplasts contract sharply and swiftly from the cell walls, because of the absorption of water by the alcohol; the protoplasmic masses become hard and even brittle; and they appear less translucent and more granular. All evident motion, except the contraction and collapse, ceases at once. Neither shape nor motion can be restored by displacing the alcohol by water. The cell is dead. What has happened is the withdrawal of water, the destruction of turgor and hence of the mechanical system of the cell, the extraction and solution of the fatty and other substances of cell and protoplasm which are soluble in alcohol, and the coagulation (precipitation) of the proteins. The coagulation thus produced by strong alcohol has the same cause as the coagulation which we saw produced by high temperature (p. 11), namely the withdrawal of water from the proteins, and is equally irreversible. We shall come back to this again (p. 152).

The effects produced by Absolute Alcohol can be produced by lower and lower percentages of alcohol. If one test the results of the application of a dilute alcohol—say 2½%—one will note that there is no contraction; that there is little or no change in color, translucence, or granular appearance; and that the rate of movement of the protoplasm is increased. If there is no contraction, there is no withdrawal of water; if there is no change in color, translucence, or granular appearance, there is no withdrawal of other substances; and the accelerated motion implies the entrance of alcohol molecules into the cell in sufficient numbers to be stimulating or irritating. The chemistry of this stimulation is obscure, but it is interesting to be able to observe its actual occurrence. The speed of movement of protoplasm increases up to a certain point with increase in the amount or percentage of alcohol; but beyond this point the speed declines until, on the addition of strong alcohol, the movement stops immediately after the alcohol reaches and enters the cell. Between coagulation by concentrated alcohol, due to the withdrawal of water from the protein constituents of the cell, and the quickening of the protoplasmic currents by dilute alcohol, due to unknown causes,[1] there are all gradations both in the same species and among different species.

These observations under the microscope parallel human experience with alcohol. We know that alcohol of different degrees of concentration produces profound nervous effects; that these are greater in some individuals of the same race than in others; that some races are more sensitive to alcohol than are others; and that these individual and racial results collect into very grave social problems. Accurate and penetrating observation, bold and skillful experiment upon living cells under the microscope, and unprejudiced conclusion from the facts ascertained, lead to convictions about the uses of alcohol which are more valuable than any number of sentimental wishes. The danger in freely drinking a substance which has the chemical and physical, and therefore physiological, properties of alcohol is obvious. The safest way to deal with poisons is to avoid them.

---

[1] It may be suspected, with fair evidence in favor of this view, that the stimulating and narcotic effects of alcohol are due to its being a fat solvent.

When one applies an alcoholic solution of iodine to cut or abrasion one subjects the exposed cells to the action of both the alcohol, as above, and the iodine. It is well known that iodine turns starch blue, blue-black, or black, according to its concentration; it turns proteins yellow, and may therefore be used as a diagnostic test for the presence of nitrogenous carbon compounds; and it leaves cellulose, the fats and oils, and the mineral matters, whether constituents or merely enclosures, unchanged in color; but the movements of protoplasm cease, and the protoplasm presently disorganizes. These facts are disclosed by applying Tincture of Iodine, greatly diluted (say 1:20) with 35% alcohol, to living cells under the microscope; but because water is less violent in its action on living cells than alcohol, it will be more instructive to use an aqueous solution of iodine.

The iodine solutions commonly used in botanical laboratories consist of potassium iodide (KI) 5 grams, iodine (I) 1 gram, and 500-1000 cc distilled water. Nothing need be said now as to the nature of this solution, though this will be discussed later (p. 36). But one should first recall what one has already learned about the effect of water on cells (p. 12), and then determine experimentally the effect of a 1% or ½% solution of potassium iodide, before attempting to ascertain the effect of the iodine. While potassium iodide solutions have the well known effect of stimulating the secretions of the mucous membranes of eyes, nose, and throat, I have not observed any change in the rate or direction of protoplasmic movement when KI is applied to such living cells as those used in our experiments. Nor does there seem to be any other visible change following the substitution of a dilute solution of KI for water. We are, therefore, now able to determine what the visible effects of iodine itself may be.

As is the case with alcohol, iodine will act as a poison or as a stimulant, according to the concentration of the solution which carries it. The usual laboratory solution is quickly fatal to living cells, but the appearances are very different from those when cells are killed by alcohol. This is partly due to the ameliorating effect of the water—there is no dehydration of any sort; partly due to the changes in color following the absorption of iodine; and partly to the fact that the iodine does not act as a solvent, but chemically combines with some of the cell constituents. Protein iodides and other iodine compounds form. These more or less pronouncedly alter the composition of the cell as a structure. Some of these freshly formed iodine compounds dissolve in the cell sap or in the outside water. In this way the mechanical arrangement of the protoplasm becomes altered. The protoplasm presently begins to disorganize, drawing or falling away from the walls, and breaking up, still surrounded by the cell wall, into irregular lumps of granular and yellow-brownish, obviously nitrogenous, materials. There may be other granules also, uncolored, and these we can demonstrate to be insoluble mineral particles (p. 27). They may be regarded as by-products in the nutrition of the protoplasm, or as waste products excreted in insoluble form, or as present at all merely because, being present in the environment of the plants under examination, they necessarily come into their cells and are there precipitated. We shall further examine this later (pp. 27-8).

The brown granules are aggregated insoluble nitrogenous compounds of carbon. The clear yellow-brown material is much more finely divided and hence more uniform in size. Both must be extremely complex iodine-carbon-nitrogen compounds.

If one apply a very dilute iodine solution—say 0.01% in water—to living cells with moving protoplasm, there may be some slight changes in color along with the accelerated movement, but their appearance will not be altered otherwise. The acceleration of movement is due to chemical causes at least as unknown at present as the chemical basis of the visible effect of alcohol on living cells (p. 15).

The range of concentrations of iodine between merely stimulating and promptly fatal will produce a range of effects accurately corresponding. So pronounced are these effects that one questions the wisdom of the undiscriminating and unknowing application of this and other antiseptics to human tissues. Without doubting the value of iodine when it is needed, as stimulant, counter-irritant, and antiseptic, one may well doubt the advisability of applying it unless one knows that it is needed and in which rôle.

Carbohydrates. The presence or absence of proteins and starch being proved by the treatment of living cells with iodine solution, we can proceed to examination of the protoplasm for further substances. Starch being a carbohydrate, insoluble in water as is its isomer cellulose, we may regard the two as products of the cell, of the protoplasm, rather than as constituents of it. A rough test of the presence of other carbohydrates in the form of sugars in the living cell, and as constituent parts of the protoplasm, can be made under the microscope, with material of the same sorts as previously described, and with many others. If a few drops of Benedict's Solution [1] be applied to the cells on a slide, and the slide heated over a water bath for about five minutes, a granular precipitate will form in the cells if sugars are present. If abundant enough, the red color indicating a reducing sugar, may be seen. Generally speaking, however, microchemical tests of sugars in living cells are unsuccessful. Sometimes the application of moderately concentrated sulphuric acid will produce a rose-red color in protoplasm before blackening takes place. This is due to the presence of sugar in the protoplasm, whether as contained product or as constituent of the protoplasm this test does not show. One must have recourse to the macro-methods of biochemistry to determine this, and reference is made again to Morrow's book.

---

[1] From Morrow, C. A., *Biochemical Laboratory Methods,* p. 177, 1927. "Dissolve 173 oz. Sodium citrate and 100 g. anhydrous Sodium carbonate in 600 cc of distilled water by heating. If necessary, filter the solution through a fluted filter paper. Dissolve 17.39 g. of copper sulphate in about 155 cc distilled water.

Lipoids. Other fat solvents than alcohol may be applied to living cells, still further elucidating the matter of oily or fatty or *lipoid* contents and constituents of living protoplasm. It is necessary to employ such substances as will mix with water. Gasoline, benzene, and other common liquids used to "cut" grease, are inadvisable. Chloroform and ether, on the other hand, which dissolve somewhat in water, can be brought into direct contact with living cells, and can, therefore, be readily applied to them in any desired concentration. Tap or distilled water, saturated by shaking with chloroform or ether, and applied to the usual material, will stop the circulation of the protoplasm, and this will presently be followed by its disorganization. The same results will follow the application of chloroform or ether directly; but if one apply only a dilute solution of either, the speed of protoplasmic movement will be increased; ether and chloroform in small amounts acting as a stimulant. This corresponds to human knowledge and experience, as in the better known case of the effects of alcohol (p. 16). Chloroform and ether, extremely useful anaesthetics, fatal poisons in over dose, are also nerve stimulants in small doses, and are among the dangerous "habit-forming"

Pour the carbonate citrate solution into a large beaker, and add the copper sulphate solution slowly with constant stirring; then dilute to 1 liter. The reagent is ready for use and does not deteriorate upon long standing."

drugs. Under the microscope, they are seen in due concentration to bring about the same appearances as strong alcohol; namely, the stopping of protoplasmic movement, the shrinkage and disorganization of the protoplast, decrease in translucence and increase in granular appearance. Other changes, to be considered later in connection with the absorption of water and dissolved substances, and resulting in changes in permeability of the protoplasmic membranes (see p. 39) also take place, but they are not directly visible. Color changes in the chromatophores or in the cell sap may also occur, as the pigments are generally soluble in fat solvents.

The presence of fat or oil as reserve non-nitrogenous food, in droplets or small solid masses, can be demonstrated by staining the cell with alcoholic solution of alkannin,[1] or with very dilute solutions of osmic acid (0.1-1.0%) in distilled water, which blackens the lipoids, but also the tannins if any are present. Several other methods are also useful, but they are not necessary here.[2] Discussion of these reserve foods will follow (p. 96).

Salts. The calcium, potassium, and magnesium salts of nitric, sulphuric, and phosphoric acids constitute the principal mineral ingredients of living protoplasm, as they do of every nutrient solution, whether artificial or occurring in Nature in the soil, in ponds, lakes, the streams, and the sea. To realize the presence of these and other mineral matters in living cells it is only necessary to recall that plant and animal bodies, when completely burned, always leave an ashy residue. Hence the name "ash constituents" for these mineral matters. That they are indispensable food materials is shown by the nutrition studies of animal and plant physiologists (p. 74). Other mineral matters are also present, whether because they are essential, or because they are in the environment in soluble form and hence are inevitably absorbed, is not always clear. Common salt, NaCl, is almost everywhere, in living cells as well as outside, and it appears to be required by the higher animals; but a minimum at least of manganese, boron, and other elements, far less universally distributed, appears to be necessary for healthy life of many plants. Quantitative analysis reveals the presence of all of these elements in protoplasm; but to demonstrate their individual presence in living cells under the microscope, as in the case of the proteins and lipoids (pp. 15-20), is not now possible. Biochemical methods must be resorted to for further information.

---

[1] The red coloring matter of the roots of *Alkanna tinctoria,* which stains fats, oils, and resins red.

[2] There is no modern book in English on botanical microchemistry. There are many books on biochemistry, of which perhaps Morrow's, already referred to, is the best for our purposes. For microchemistry the student is referred to Molisch, H., *Mikrochemie der Pflanzen,* 3d ed., Jena, 1923, Tunmann, O., *Pflanzenmikrochemie,* 2d ed., Berlin, 1931.

The rôle of the mineral salts in living cells, living protoplasm, has been much discussed; but from our present point of view, we must recognize, among other uses, their importance as carriers of electrical charges and currents. The name, electrolytes, is significant. As components of a system consisting of proteins, carbohydrates, and lipoids, which are all relatively inactive electrically, the electrolytes and the water, electrically very active, may evidently be of the utmost importance because of their electrical properties. Furthermore, the proteins, carbohydrates, and lipoids do not dissociate in dilute or other solutions. The electrolytes, on the other hand, as their name implies, may be dissociated by electric current, and they regularly dissociate in dilute aqueous solutions.

From the foregoing experiments (p. 13) and from those following (p. 47), we realize that while the cell sap is a solution somewhat more concentrated than that of the natural waters, from which it absorbs, it is still a dilute solution, in the sense of the physical chemist. In it, therefore, in the vacuoles of the cell, and throughout the protoplasm, the electrolytes are present both as compounds (salts) and as ions (elements or groups of elements). This greatly increases the possibility of their activities in every way, both physically and chemically, contributing to the capacities of a cell as a unit and of any and all of its constituent parts and contents. Because of the extraordinarily varied qualities of the alkaline elements, calcium, potassium, and magnesium, and of the acid radicles in combination or association with which they enter living cells, the electrolytes obviously offer a great range of possible chemical combination within the boundaries of the cells of which they are parts or inclusions. It would be going too far to say that the visible movements and the other activities of living protoplasm are due to the electrical and chemical possibilities afforded in the cell by the presence of these extremely active ingredients of the living protoplasm; yet one will not hesitate to say that our present very imperfect understanding of life, living, and living substance is greatly helped by increasing knowledge of these inorganic chemical compounds.

Protoplasm is often spoken of as labile, i.e. liable to chemical change. This lability must be due, to a great extent, to the activities of the electrolytes (p. 7). Protoplasm is extremely sensitive to external influences, as shown above (p. 10), as we know by experience, and as we shall see later (pp. 132-59). In the occurrence of dissociated ions of extremely active elements and radicles with unstable, unsaturated proteins, carbohydrates, and lipoids, in such a medium, water, as promotes movement and other activities, we see some of the reasons for the extraordinary, the unique, properties and potentialities of living things.

The application of other reagents and stimulants to living cells under the microscope will lead to more precise and adequate ideas about living protoplasm, its structure and components. In Nature there are rocks which give to the soils and waters of certain localities, particularly in the far west, qualities which are called alkaline. Other soils are said to be acid. It becomes us therefore to observe the effects of alkaline and acid solutions of different concentrations upon living cells. In ordinary experience one observes that, whereas brief exposure may produce no evident response to a small amount of stimulation or a small quantity of stimulant, prolonged or frequently repeated exposure may produce profound results. In agriculture, in horticulture, and in Nature, cumulative effects are observed which seem to be out of all proportion to the quantity of the stimulant or irritant. We shall the better conceive, though we may not understand, this if we study the effects of alkalis, acids, etc. upon living cells under the microscope.

Alkalis. Sodium, potassium, calcium, and ammonium are known as akalis. Their compounds are more or less alkaline, according to the acid radicle with which they are united. They may be quite neutral in compound, but when the compound dissociates the separate action of the acid and the base may be very destructive. Experiment may be made to show this, thus: if a saturated solution of sodium or potassium hydroxide be applied to a stamen hair or squash hair, or to an *Elodea* leaf, or to other living cells, there may be some shrinkage of protoplasm from plasmolysis, and there will be some yellowing, but the effects will not be very marked. On the other hand, let one substitute distilled water for the saturated sodium or potassium hydroxide solution by drawing the water under the cover-glass by placing a strip of filter paper on the side opposite the one on which the water is added. The results of this operation are the reversal of whatever plasmolysis was produced by the saturated solution and subsequently, as the dilution is increased, the solution of all the contents of the cell but the mineral particles. The walls are less affected by dilute than by concentrated alkaline solutions, but the starches dissolve and the lipoids become saponified in solutions of various concentrations, while the proteins go into solution only as the alkaline solutions become more and more diluted. One may infer, therefore, that the protoplasmic structure and its components are not attacked by NaOH or KOH molecules, but that as soon as the solution becomes diluted enough for the Na and

OH or K and OH ions to dissociate, enough of the constituent proteins combine with the extremely active sodium or potassium to form soluble protein compounds. The dissolving of these sodium or potassium "proteinates" breaks the system and destroys the structure, which presently disappears, with only the insoluble mineral particles remaining as visible granules. The dancing of these in the yellow liquid is an example of Brownian Movement. It may be used to measure the viscosity of a fluid.

The behavior of living cells under the influence of the far less active alkali, calcium hydroxide, is much less striking, but the volatile ammonium hydroxide approaches the sodium and potassium solutions in its effects. The color produced is more greenish yellow. If very dilute ammonium hydroxide solution is applied to living cells, the ammonia stimulates the protoplasm, as shown by speedier movement. The chemistry of this effect is, at present, only the subject of scientific guesswork. If one determine the acidity or alkalinity of the cell sap and of the protoplasm of living cells, one will find that the cell sap is slightly acid (pH 5.6) and the protoplasm slightly alkaline (pH 5.8). This difference appears to be very uniformly maintained under many varied conditions; but if one alter the surrounding medium, water or air, as to its reaction—"hardening" or "softening" the water, polluting the air with acid fumes[1]—manifestly the reaction of cell sap and protoplasm will be affected, the relation of the pH of the one to the pH of the other will be changed. The magnitude of this change, and its promptness and duration, depend upon many factors, but among them are the properties and the amount of the reagent employed to produce the change. This change in reaction in both cell sap and protoplasm affects every chemical relation and reaction within a living cell, showing itself in the above recognized change in rate of protoplasmic movement.

Dissociation. Furthermore, according to the current views in physics and biochemistry, the materials in protoplasm in the living cell, and in its sap, are in the various states of solution, dissociation, suspension, and emulsion, according to the various materials and the common solvent, water. But water itself is no longer regarded as simple, wholly a compound of hydrogen and oxygen in the familiar proportions of H_2 and O. On the contrary while the major part of it may be H_2O the minor part, variously estimated in amount according to time and circumstances, consists of the dissociated hydrogen (H) and hydroxyl (OH) ions. As hydrogen is characteristic of acids, and the hydroxyl group of alkalis, their ions carry positive and negative electrical charges respectively. It is evident that pure water will contain equal numbers of hydrogen and hydroxyl ions, and its reaction will therefore be perfectly or absolutely neutral. Any change, however, in the proportion of hydrogen and hydroxyl ions in a given volume of water, by adding a soluble substance to it, will at once cause the solution to become correspondingly acid or alkaline.

---

[1] *The Physiology of Plants,* pp. 83-7, 144 ff.

This can and should be indicated by experiment, the more so since the preparation of absolutely pure water is so extremely difficult that one is forced to realize that it never long exists anywhere in Nature, and certainly never in living cells and living organisms. By using one of the usual colorimetric sets for determining pH values, one may ascertain the "hardness" or "softness" of tap and other waters and the reaction of the laboratory distilled water. In a laboratory in which gas is used for heating purposes, and in which chemicals are employed, *pure* distilled water cannot exist; for the water will take up, dissolve, from the air acid and alkaline ions so that it cannot be *perfectly* and exactly neutral. Cell sap, i.e. the water in a living cell, of plant or animal, is therefore water consisting of H_2O molecules and of H and OH ions to which acid, hydroxyl, and many other ions are added from the protoplasm and by its activities, and from the surrounding air, water, etc. As we shall presently see (p. 108), for example, the respiratory activity of living protoplasm ordinarily results, among other things, in the liberation of carbon dioxide following the intake of oxygen. By this change the cell sap would tend to be acidified, other things being equal. Other physiological processes contribute their shares of hydrogen or hydroxyl ions, or their derivatives, with the resulting effects upon and in the cell sap and the protoplasm. Hence the reaction of the cell, its pH, is an extremely sensitive thing, maintained with extraordinary accuracy, and under greatly varying conditions. In this remarkable combination of very active alkaline elements, acid and alkaline ions, and living protoplasm, we have the most sensitive and self-operating mechanism known.

Acids. We may now take the next step in attempting to understand living cells and living organisms. The effects of acids should now be determined. Acids may be classified as mineral or organic. The former are hydrochloric, nitric, sulphuric, phosphoric, etc.; the latter contain carbon, and include acetic acid, malic, citric, etc. From their effects on living cells we can draw further inference as to the composition and structure of cells, their products, and their contents. Furthermore, since many acids are found in one place or another in Nature, and we are familiar with their collective effect on living organisms, we may discover the basis of these collective effects in the reactions of the living cells under the microscope. For example, hydrochloric acid is produced by the human stomach, and seems to be indispensable in digestion. Acetic acid, under the name of vinegar, is derived from the fermented juice of many fruits. Malic and citric acids are contained, as their names imply, in apples and oranges and the allies of the latter —grape fruit, lemons, limes, etc. Oxalic acid or its soluble salts is in rhubarb, tomatoes, etc. Cranberries contain citric acid. Tartaric acid is in the juice of grapes and comes down as the calcium salt in the making of wine. Other examples might also be cited.

In many plants the insoluble calcium oxalate is crystallized out, in many other plants the soluble or insoluble salts of organic or mineral acids are present in the living cells. The acids are presumably the products of oxidative metabolism (respiration, pp. 108-21). The bases, coming into the cell as salts, or as ions associated with acid radicles of soil constituents, and needed in only small amounts as protoplasmic constituents, may combine with the oxalic acid liberated in respiration, thus forming soluble oxalates (sodium, potassium, and magnesium oxalate), or an insoluble one (calcium oxalate) which may crystallize out in the cell as needles or as massive blocks. Examples of all of these things may be seen, for example, in the fruits already cited, in Begonia stems and leaves, in the leaves of the rubber plant *(Ficus elastica)* and in many other places (see Molisch, *Mikrochemie*).

We may ascertain, therefore, from putting acids upon living cells, what the composition, or at least the contents, of these cells may be in certain particulars. Thus if we apply dilute oxalic acid (not over 5%) to living cells we should expect the precipitation or crystallization of calcium oxalate, if calcium is present in forms or amounts thus to become evident. The methods of microchemical analysis enable one to draw significant conclusions from the results of applying acids—and correspondingly of applying alkalis—to living cells, especially if the cell or the cell sap contain coloring matters at all sensitive to changes in the reaction (pH). Thus if the purple or pink stamen hairs of *Tradescantia virginica*, the "Spiderwort," or of *T. zebrina*, a colored "Wandering Jew," be treated under the microscope with dilute hydrochloric acid, one per cent or less, the change in color of the cell sap will indicate at once the influence of the acid. By using still greater dilutions, of known strength, on a series of specimens on separate slides, one can ascertain the concentration of acid which most nearly approximates the point at which no change in color is shown. From this one may infer that the acidity (or pH) of the cell sap is approximately that of the solution used.

Instead of using percentages, one may with greater accuracy employ so-called *normal solutions* in which the number of grams of solute contained in a liter of the solution is indicated by the molecular weight, or fractions thereof. Thus a normal solution of hydrochloric acid is one which contains 36.18 grams per liter. This is a 3.618% solution. Instead, however, of saying a 3.618% solution, and trying to make such a one, we may speak of normal solutions and make them accordingly, using $\frac{1}{10}$ N, $\frac{1}{100}$ N, or $\frac{1}{1000}$N or any similar fraction that we please.[1]

To confirm the conclusion above as to the pH of the cell sap of the cells as indicated by HCl, we may use other acids in similar fashion, bearing in mind that other reactions than the color one are undoubtedly involved, as previously indicated by the treatment with oxalic acid (p. 28). Sulphuric acid will undoubtedly form sulphates, some insoluble, most of them soluble. Nitric acid will form only soluble salts. But acetic, osmic, chromic, and picric acids, in common use in cytology, form insoluble protein compounds. By so doing, even in solutions as dilute as 1%, they "*fix*" or *set* the protoplasm in the positions which it occupied when the reagent reached it. For this reason they are in general use and highly valued. Their operation may be followed under the microscope, and much may be learned of the behavior of the protoplasm of the cell as a whole and as to its component parts.

Structure of Protoplasm: Colloidal State. To infer from the appearance of a fixed cell that protoplasm has a structure, in the mechanical sense of being a froth or a tangle of fibrils as Bütschli supposed, is unnecessary and seems now unjustified. On the basis of investigations of bio-chemical and bio-physical-chemical nature, one is at present much more disposed to conceive protoplasm, nucleus, chromosomes, genes, and all the other constituents of living cells, in the terms of molecules, atoms, ions, bearing corresponding electrical charges, and built up into a system or systems consisting of water and other substances. Just as, in water, we may have pure water consisting of H_2O molecules, and H and OH ions, so we may have other substances dissociating (see p. 22); but one may also have aggregations of various sizes, resulting in what is known as the *colloidal state* of many of the cell components.[1] In a solution, one has the molecules of the dissolved substance separating from each other as far as the space limits permit, and becoming uniformly distributed throughout the volume of the solvent. In the colloidal condition, no such division and separation into molecules, and even ions, takes place. On the contrary the molecules are in larger or smaller groups. The solvent and solute or solutes of a solution pass through ordinary filters, whereas materials in the colloidal state, in a suspension which may be as translucent as a solution, will not pass through the pores of a fine filter. Furthermore, if a beam of light is directed upon a solution it will pass through, without revealing any structure in the solution; whereas, directed upon a suspension of a solid in colloidal condition, the beam of light will be diffused, and the particles may even be distinguished by the eye, or by the ultra-microscope, or by dark-field illumination, as before described (see pp. 6-7). This is known as the Tyndall effect, from the studies of the great British physicist of the last century, John Tyndall. He observed, and explained, the motes which one sees dancing in beams of light admitted into a dark room through small openings.

---

[1] The principal reason, however, for employing "normal solutions" is that they indicate the number of molecules in the solutions, and are therefore comparable quantities; whereas, without calculation, one cannot tell the numbers of molecules concerned in percentage solutions.

We can now understand why protoplasm cannot be filtered. It may be separated from an excess of water, for example, by being filtered out, but the protoplasm itself cannot be divided by filtration, as one can separate a liquid from the precipitate in it. The colloids, of which protoplasm in part consists, hold the rest of its constituents, even when considerable pressure is applied to force them out. We can squeeze out a liquid, called press-juice, and by the usual methods we can determine its pH, composition, etc.; but as we wreck the protoplasm in the process, stopping some of the reactions which contribute to maintaining that balance of destructive and constructive operations in living cells which we call life or living (see *Physiology of Plants,* p. 7), we should not feel sure that the results obtained indicate the actual condition in the living cell. Indeed, real knowledge of the actual condition in any living cell is a matter of scientific inference from imperfectly observed and conceived fact. Thus we can determine the pH of cell sap *extracted from* living cells; by the method described above, of applying dilute acid to colored living cells, we can determine the pH of the cell sap *acted upon by dilute acid;* but we have no means, at present, of ascertaining the pH really prevailing (or changing) in actively living cells, for no cell is the same as before we touched it!

---

[1] Findlay, A., *Physical Chemistry for Students of Medicine*, pp. 170 ff. New York, 1924.

The colloidal condition in which many of the constituents of protoplasm exist in living cells leads, therefore, to a conception of protoplasmic structure quite different from that current two decades ago; to a conception of the mutual relations of the components of this system of water, colloids, electrolytes, carbohydrates, and lipoids which leads directly to a perception of why and how such a system is so full of activity. What balances and coordinates the multifarious actions and reactions taking place in a living cell is not yet revealed, though in the pH of the cell sap, whether changing or uniform, we see one regulatory influence.

Antiseptics. Certain substances are in such general as well as professional use as antiseptics that we may well apply them to living cells and observe their visible effects under the microscope. At the present time iodine, in alcoholic solution principally, is the antiseptic most commonly applied to cuts. Its immediate effects and the subsequent changes we have already examined (pp. 15-19). From our observation of its effect upon one set of cells, which may also be subject to attack by other and parasitic cells (bacteria of disease), we are forced to conclude that it is hard to exaggerate its value as an antiseptic when an antiseptic is needed, but that used at other times it acts only as an irritant. If a counter-irritant is needed, as at times in the mouth, or upon a bruised muscle, the irritant or stimulant effect of iodine is desirable; but burns may only too easily result, as experience has shown to many persons. Iodine, applied in order to kill bacteria, may also kill the cells of the exposed or adjoining tissue. In this case the cut or laceration, and the poisonous effect of the iodine must both be healed, a double task both parts of which may have been equally unnecessary. In other words, the practice of domestic medicine involves actions not to be undertaken without proper consideration of the consequences.

Mercurochrome, hexylresorcinol ("white mercurochrome"), carbolic acid in 0.5% and 3% solutions, 1% formalin (too commonly used as a preservative), 3% silver nitrate, 3% peroxide of hydrogen, 0.5% potassium permanganate, "chloride of lime," and any other antiseptics, germicides, and preservatives may be applied to living cells and their visible influence, as indicated by change in movement, or color, or arrangement, valued as significant evidence of the sensitiveness, chemical activity, and plasticity of the living substance, protoplasm.

Alkaloids, etc. We may similarly apply the common stimulants, cocaine, caffeine, nicotine, to living cells and watch the visible effects. These consist most conspicuously in changes in the rate of movement of the protoplasm; but, since the movement must depend upon chemical action, change in the rate or kind of chemical activity in the cell plasma will necessarily affect the rate of movement. Or, *vice versa*, we must conclude from change of movement, the chemical activity of the cells is changed.

As we shall presently see, the chemical activities of the cell generally fall into one or another of two categories. Either they are constructive, building the body in growth or repair, making food, etc.; or they are destructive, supplying the body with energy for growth or repair or doing work, burning food, etc. Collectively these processes are called metabolism. From their effect upon the rate of protoplasmic movement, as seen under the microscope, one concludes that these stimulants, and also many others such as alcohol (pp. 15-21), affect metabolism, thus acting upon the nerves of more highly organized organisms.

CHAPTER II

ABSORPTION

The living cells and living organisms studied by the methods indicated in the preceding chapter, are formed from raw materials taken in from outside. These raw materials are parts of the environment, of the world we live in; they have their places in Nature as parts of the air, the land, and the sea or the streams. While we commonly speak of them as not only lifeless but inert as well, they may nevertheless engage in various operations through the exercise of their own kinetic energy. For example, inert matter may be motionless in air, but in water it dissolves, its molecules move (diffuse) in all directions, locomotion actually takes place, even though there may be obstacles in the path.

Diffusion. We may refresh our knowledge of the phenomena of solution and diffusion by purely qualitative experiments, making these quantitative as occasion and desire indicate; and then apply this information to our consideration of living things. If one grind a small quantity of table sugar in a fine mortar until the particles are reduced to the smallest possible size, examining it from time to time on a slide under the microscope, one will see granules of perfectly visible proportions. If now one draw a drop of distilled water into contact with a particle, the particle of sugar, of the smallest size possible to reach by grinding, is seen to dwindle, it seems to be eaten off by the water, and it finally disappears. Of course it can be wholly recovered again by drying off the water; but meantime the sugar molecules, apparently motionless in air, leap off into the water, moving of their own energy of motion (kinetic energy) throughout the drop of water. The same phenomenon will be exhibited by other familiar substances.

Such soluble colored substances as potassium bichromate, potassium permanganate, copper sulphate, anilin dyes (eosin, gentian violet, acid fuchsin, light green, etc.) and a substance of such regular crystalline form as common salt, if treated in the same way as sugar, will all show that the solid particles diminish in size when water is brought into contact with them. The color of the dyes, etc., will presently become uniformly distributed throughout the drop or volume of water; but the colors will not diffuse at equal rates, nor will the rates of solution and of diffusion be the same. By using calibrated tubes of uniform diameter one can measure the rates of diffusion of different dissolved substances and thus confirm and extend the conclusions formed from microscopic observation, both qualitatively and quantitatively.

As we have previously seen (p. 26) that water in Nature always holds other substances in solution, our experiments under the microscope and otherwise with ordinary distilled water must be supplemented by experiments with solutions. If one have one drop of 10% sugar solution and one drop of 1% sugar solution on a slide under the microscope they will completely unite and mix if merely brought into contact with each other. Furthermore, particles of sugar will dissolve with approximately the same speed in distilled water and in the 1% and 10% solutions. This is not true, however, of all substances or all solutions, as may be shown by bringing drops of 5% salt solution into contact, under the microscope, with particles of the various colored substances named above. It will be seen that the salt *delays* the solution or the diffusion of one or another of the substances used.

Solution and diffusion may be *hastened* by substances already in solution. One of the prettiest experiments to prove this may be made by placing a white saucer of distilled water, or a watch glass or other convenient glass dish of distilled water, over white tile or white paper, and dropping a flake of solid iodine into it. Examination will show only the slightest change in color, or other evidence of solution and diffusion; but if one now drop a crystalline mass of potassium iodide into the water, on the side opposite the position of the iodine, one can observe the solution of the salt, the rapid spread of the solution in all directions, and very shortly the putting out from the flake of iodine of what resemble brown streams, flowing mainly toward the potassium iodide. One can measure and time these streams. One can modify their direction by placing other pieces of potassium iodide in other parts of the solution. The iodine which is only very slightly soluble in pure water, dissolves and diffuses rapidly in potassium iodide solution.

On the other hand, if one place a piece of barium hydroxide on one side of a watch glass of distilled water set upon black tile, and a drop of strong sulphuric acid upon the opposite side of the water, the barium hydroxide will dissolve and diffuse promptly in all directions, as will the sulphuric acid also. Where the sulphuric acid and barium hydroxide meet a precipitate of barium sulphate forms. Thus one substance in a solution may, by uniting with it, remove another substance from the solution. If there is a surplus of one, there may be complete removal of the other. The removal, or precipitation, occurs at the line of contact, or the interface, wherever this may be. This is true of all chemical changes: they occur at points, lines, surfaces, or interfaces, of contact.

As we have seen crystals of sugar and of salt taken to pieces, and made smaller and smaller by distilled water, until they were reduced to particles invisibly small (p. 34), so we have seen matter reduced to particles with larger and larger surfaces in proportion to masses. The process of solution, therefore, not only makes matter motile (diffusible), but it enables it to engage most readily in whatever chemical changes are possible at the prevailing temperature and pressure.

Membranes. Living cells differ, however, from the solutions used in the above experiments in being separate individual units: they will not fuse or merge or otherwise unite, except under the very special conditions of reproduction (p. 160), although we can make two drops of water or two drops of aqueous solution unite by bringing them into contact. Whereas we can conceive an interface between air and water or water and oil, we cannot conceive an interface between water and water. Nor can we conceive an interface between naked protoplasm and naked protoplasm. The two will fuse, as water merges with water. How, then, can we account for the familiar fact that cells, organs, organisms are individual discreet objects?

In cells provided with visible cellulose walls, such confluence as occurs where an interface is not formed, evidently cannot take place. The cellulose wall is a membrane containing much or little water, according to the proportions of its other contents; but it is structurally so different from the cell which forms it that it cannot merge with the protoplasm. But we must realize that the water within a cell, in its protoplasm and in its cellulose, is continuous with the water outside. The water inside and outside form a continuous mass, just as we can suspend a colander in a pan of water and have the water continuous, above, below, and through it. But however perfectly continuous the water in a pan may be, two colanders which one may put into the same pan will remain separate from each other as discrete entities. So protoplasms remain distinct, discrete entities; but not merely because of their cellulose walls, for we have seen (p. 13) that the protoplasm is bounded by membranes, both external and vacuolar. These membranes also contain much or little water, according to circumstances, and constitute therefore other colanders around the protoplast. These cytoplasmic membranes, being weaker but very much finer in every other respect than the comparatively coarse cellulose walls, enclose parts of the mass of water which is continuous throughout the body of the organism, but the water strands through these cytoplasmic membranes are much too fine to permit the passage of every sort of substance.

Impermeable Membranes. Only a film of glass or some such material is perfectly impermeable, but in the purple or pink cells of *Tradescantia* stamen hairs (see pp. 6-28), in the red cells of table beets, in the blue and red petals of many flowers, and in many other places, water-dissolved pigments are perfectly held within the cells which form them, although the cells take up water freely. If one puncture such a cell under the microscope, as may be done with a sufficiently fine needle, it will be seen that the color will escape through the wound. If one could puncture only the cell wall, the color would not escape. If one kill the cell by heat or by any other means, the color will escape from the central vacuoles, diffusing outward in all directions. Between the escape through a mechanical injury, a rupture or puncture, and that by diffusion from the central vacuole, there are many differences, though the one most interesting at the moment is that death is followed by, perhaps due to, a difference in permeability. The vacuolar membrane—the cytoplasmic membrane surrounding the vacuole with the colored cell sap—is impermeable to the pigment during the life of the cell, but becomes permeable to it when the cell is heated or otherwise injured.

Semi-Permeable Membranes. Living cells are, therefore, bounded by membranes, invisibly thin, which permit the free passage of certain substances in solution but exclude other dissolved substances. Many names have been proposed for such membranes. No single name, however, conveys the exact meaning; for a membrane completely and perfectly permeable to water and certain dissolved substances and impermeable to other substances in solution in the water, is much more than semi-permeable, or partially permeable; it may be said to be selectively or differentially permeable, except that whether a given substance will go through or not is an attribute of the substance rather than of the membrane. This attribute apparently consists in the size and shape of the molecules of the substances. Thus substances with small molecules like the salts, pass through easily; whereas substances with such large molecules as the carbohydrates, or with still larger molecules, like the proteins, or with molecules aggregated as in the colloidal state, pass through membranes more slowly if at all. This can be shown by applying a 4% potassium nitrate solution in distilled water to living cells under the microscope. The cells will shrink because more water leaves the cells than enters them, owing to the difference in the concentration of the cell sap and the KNO_3 solution. But if one use KNO_3 solutions colored by methylen blue and by eosin, one will see that these do and do not enter the cells respectively. The explanation is that the molecules of methylen blue are small in comparison with the molecules of eosin. The latter cannot get through the cytoplasmic membranes of living cells. But if, as before (p. 11), the cells are killed by heat, the eosin will promptly enter and stain the cell.

Osmosis. The passage of dissolved material through a membrane, diffusion through a membrane, is known as *osmosis*. It is due to the kinetic energy of the molecules of the dissolved substance itself, in other words, to the pressure of the molecules of the dissolved substance upon the barrier. In the case of a rigid barrier like a bottle, there is no evidence of this pressure. The molecules strike the glass wall of the bottle; they rebound: the glass resists, or takes up, or absorbs the blow: the glass itself contains no water. There is no passage through it. On the other hand, our colander or membrane is also struck by the molecules of the substance moving freely throughout the solution; but the membrane is porous, and molecules which enter the water-filled pores will move as freely as in other parts of the volume of water, even passing through the pores to the other side. This is shown by the usual so-called osmometers. Of these, probably the most convenient form is the so-called diffusion shell or diffusion thimble. This consists of filter paper pressed into cup or thimble form and treated with concentrated sulphuric acid to convert it into "vegetable parchment," a tough semi-permeable material. If such a cup, conveniently full of dilute sulphuric acid, is suspended in a beaker of barium hydroxide solution, one will presently see that white barium sulphate is being precipitated as the sulphuric acid passes through the membrane of the cup into the barium hydroxide solution.

If there are equal numbers of molecules of a given substance in solution on opposite sides of a passable barrier the pressures on the two sides will be equal, and the movements of the molecules in opposite directions through the barrier will be equal. But if the numbers are unequal, then the pressures will be unequal and the movements will be correspondingly unequal, for molecules move, like other things, in the direction of least resistance. Hence, if there are fewer molecules of water, or of a given substance dissolved in it, in a cell surrounded by its bounding but semi-permeable membranes, molecules of water or molecules of the dissolved substance will move through the membrane and into the cell. This is what absorption is, and it can be illustrated very easily by diffusion shells, thus. A diffusion shell nearly full of molasses and suspended in a beaker of tap water will presently overflow. A diffusion shell filled with clear barium hydroxide solution, or with dilute sulphuric acid solution, and suspended in a beaker of the other solution, will presently be found to contain a solution in which barium sulphate is being precipitated. Care should be taken to determine, by ascertaining where the precipitate first appears, whether the sulphuric acid or the barium hydroxide moves the more rapidly through the membrane, and whether this corresponds with the rates of diffusion as discussed previously (p. 35).

Absorption will, therefore, take place according to the scarcity of a given substance within a cell in comparison with the supply outside, provided there is contact between the cell and the supply (see p. 49), and provided the permeability of the intervening membranes.

Partial Pressure. From the foregoing discussion it is obvious that a substance in a solution exerts a pressure due to the kinetic energy of its molecules. This pressure is characteristic of the substance, as well as proportioned to the quantity of the substance. Thus equal numbers of molecules of sugar and salt exert unequal pressures. If both are in solution in the same volume of water, each will exert its own characteristic pressure at the same time and in the same space as the other, and the pressure of each substance is called the partial pressure of that substance in the solution. The total pressure, or the concentration, of a solution may be made up of the pressure of a single dissolved substance or of the partial pressures of several or many dissolved substances.

Isotonic Solutions. Furthermore, one may have two solutions of equal total pressures, or concentrations, but of very different compositions. Such solutions are said to be isotonic with each other. They may exchange solutes with each other and still maintain their equal pressures; or one solution, by acquiring one of the solutes of the other solution, may so increase its concentration that it will acquire water also from the other solution. In fact this sort of thing is going on all the time in Nature; for an organism, by taking in solutes (salt, for example) from outside, or making solutes (sugar, for example) within its cells, or by losing water by evaporation, perspiration, etc., so increases the concentration of its cell sap from time to time as to insure the absorption of water, until cell sap and outside water become isotonic. We can show this readily under the microscope; for if we make a camera drawing of the cells of a small section of root, stem, or leaf mounted dry under a cover-glass on a slide, and then add tap-water and redraw, we shall see that the cells have increased somewhat in size. Or if we supply tap-water to pieces or sections of salt marsh plants or to seaweeds, we shall see that they absorb water and expand.

Instead, therefore, of plant and animal cells drawing in water and dissolved substances, these substances move into the cells, as they would move into anything else, or in any other direction, in which the partial pressure, the proportion of water or of the individual dissolved substances, is less. By producing, maintaining, or avoiding higher partial pressures within themselves, and by correspondingly controlling the permeabilities of their membranes, living cells differ from lifeless (killed, "devitalized") ones and from all lifeless objects or systems. They continue or cease to absorb, and they may reverse the process and *excrete* (pp. 56-9).

Membranes. In the foregoing we have considered mainly the invisibly fine membranes which bound the protoplasm of living cells, and the very coarse clumsy imitation afforded by so-called diffusion shells or thimbles (p. 40). We may multiply experiments with these latter, learning more about the phenomena of diffusion and osmosis, but except as these shells throw light upon the properties of cellulose walls, they may be quite misleading as to the nature of the cytoplasmic membranes. For example, we may put a solution of barium hydroxide, glucose, and potassium permanganate into a diffusion shell and stand the shell in a beaker of water. If we test this outside water from time to time with dilute sulphuric acid, and with Fehling's or Benedict's Solution, we shall find that the barium salt and the glucose pass through the membrane at different rates. The permanganate, as shown by the eye, will be found to come through still more slowly.

Much thinner membranes, better imitating the protoplasmic membranes, are afforded by the so-called precipitation membranes of various sorts. If one drop a small mass of crystalline copper sulphate, copper chloride, or copper acetate, into a solution of potassium ferrocyanide, one will see that the salt promptly becomes coated with a rusty brown precipitate, and that this not only increases in amount but that the mass of salt becomes surrounded by a bag increasing in size rather than in thickness. Careful watching indeed discloses that this precipitation membrane becomes stretched, ruptures, and re-forms at the interface of copper and ferrocyanide solutions. This is the famous Traube's cell used by Pfeffer, Morse, and many others in the study of growth, osmotic, and absorption phenomena. Pfeffer used unglazed, porous clay cups as the strong backing for exceedingly thin precipitation membranes. After most scrupulous washing a porous clay cup was soaked in a solution of copper salt, the outside then thoroughly washed off, and the cup, full of copper salt solution, suspended for a short time in potassium ferrocyanide solution. In consequence exceedingly delicate small membranes of copper ferrocyanide are precipitated across the pores in the clay. If now the cup is emptied of the copper solution, and refilled with a solution of sugar or other substance to be tested, it will be found that the copper ferrocyanide membrane is freely permeable to water, but is practically impermeable to substances dissolved in it.

One may easily use the cellulose diffusion shells described above (p. 40) in place of the clay cups, washing them perfectly, finally in distilled water, soaking in copper sulphate solution, washing the outside thoroughly, supporting for a time in potassium ferrocyanide solution, and thoroughly but very carefully rinsing the outside, emptying the shell of its copper solution, refilling with, say, a dark molasses, and suspending in a beaker of water. Whereas an untreated diffusion shell permits the prompt passage of the coloring matter from the molasses through the shell into the water outside, the color is retained by a shell with intact precipitated copper ferrocyanide membranes. Such an apparatus may be converted into an osmometer by inserting a glass tube passing through a rubber stopper which may be *wired* tightly into the shell filled with the solution to be tested, for example a 1% sugar solution, 3% salt solution, 3½% potassium nitrate solution, etc. The shell is now suspended in pure water, and the intake read either in the terms of increase in volume or increase in pressure. The latter is possible if one connect a mercury manometer with the glass tube. No such pressures will be obtained with an elastic cellulose diffusion shell as with a rigid porous clay cup, but neither will great osmotic pressures (hydrostatic pressures due to endosmosis of water) develop in cells *unless* they are backed by rigid support.

As a matter of fact, the latter may be and often is attained when roots grow in heavy compact soil, between stones and bricks, or under concrete sidewalks. In these circumstances the pressures possible are, like those in the weak inner tube of a tire supported by the strong casing, much greater than the mechanical strength of the cytoplasmic membrane itself.[1]

Imbibition. Still other sorts of absorption by living organisms may be considered. If air-dry dormant seeds are put into water they will absorb water and swell. The swelling will be considerable, and it will be accomplished with such an amount of force that even great resistance will be overcome. This absorption, evidently quite distinct from the osmotic absorption treated in the foregoing pages, is known as imbibition. It can be studied, like the preceding processes, either *en masse* or under the microscope. In the latter case, if one make a reasonably thin shaving of an air-dry pea seed, place it under a cover-glass on a slide under the microscope and sketch it under *camera lucida,* one will find, on adding distilled water to the preparation, that it expands, and that it does not expand equally in all directions, and in all parts. The absorption of water, the imbibition and the swelling, are not uniform throughout the section of the seed: different substances imbibe differently. These substances may be the colloidal components of the protoplasm, or they may be carbohydrates such as starch and the cellulose of cell wall. Furthermore, if one carefully measures with micrometer calipers[1] an air-dry block of wood in its three dimensions, longitudinal, tangential, and radial, and similarly measure it again after it has lain in water for twelve hours or more, one will find its longitudinal expansion least, its radial somewhat more, and its tangential most pronounced of all. This unequal expansion in different directions is important in the preparation of the various sorts of lumber, and in the construction of wooden objects from boxes to houses.

---

[1] *The Physiology of Plants,* p. 35 ff.

It is obvious that, in the actively living condition, in which the materials of the cell and of the organism will necessarily contain all the water they require, imbibition will have taken place, and unless water be removed from protoplasm, starch, and cell walls, no more will be *imbibed,* though it may continue to be otherwise absorbed; but the force exercised by water-imbibition by dry seeds, dry wood, etc. is strikingly greater than that resulting from osmotic absorption. Imbibition develops a pressure of more than forty-one atmospheres, for example, in air-dry *Laminaria,* a seaweed;[2] but measurements of the osmotic pressures in living cells have not exceeded one-third of this pressure, enough in either case, however, to ensure the entrance of sufficient amounts of water into living organisms to start and to maintain their growth, whenever water is present in sufficient amount in the environment. Thus air-dry seeds will absorb, imbibe, moisture enough to establish themselves as living osmotic systems; and they will continue to supply themselves with water and dissolved substances, through seedling and later states, as long as they maintain themselves as living osmotic systems, by root hairs, etc.

Donnan Equilibrium. In the case of such substances as dissociate in dilute solution into ions bearing positive and negative electric charges respectively, the nature and the electric charge of the membrane and its components will determine whether both ions of the dissolved substances will pass equally or unequally through the membrane into or out of a living cell. It may be, therefore, that the unequal absorption of alkaline and acid ions of the mineral salts from soil solutions or from water (fresh or salt) is due to an electrical, rather than an osmotic or chemical, equilibrium on opposite sides of a cell membrane. This is named for the eminent English physicist who described it.[1] This may also suggest a reason for the unequal distribution of materials in cells, and in the tissues and organs of living organisms; but this requires further study and much more delicate manipulation than is contemplated here.

---

[1] Micrometer calipers of various shapes will be found useful in the laboratory and may be had of the laboratory supply houses.

[2] Findlay, A. *Physical Chemistry for Students of Medicine,* p. 199. Osterhout, W. J. V. "A Comparative Study of Permeability in Plants." *Jour. Gen. Physiol.* 1, 299-304, 1919.

The foregoing discussion and experiments make us now ready to experiment with roots to determine the force with which they can absorb water. We can thereby gain some idea in regard to the means of entrance of soluble substances also, such as the salts in the soil, and the carbon-dioxide and the oxygen of the air, which are required by most plants and animals for continued existence.

Root Hairs. If one place kernels of wheat or of the other small grains, or seeds of mustard, radish, etc. on wet filter paper or wet cloth in a dish, covering with a tumbler, germination and growth will follow within a few days. The main root will first emerge through the coats of seed or kernel. Presently at a distance of a very few millimeters behind the root tip, hairs will begin to develop. These will develop into a striking velvet covering over a considerable length of the root. In fact, the remarkable length, size, and number of root hairs is a reaction on the part of the seedling, not so much to the damp air as to the abundance of oxygen as compared with the amounts in soil or in water.[2]

---

[1] Donnan, F. G. and Allmand, A. J. "Ionic Equilibria Across Semipermeable Membranes." *Jour. Chem. Soc.* 105, 1914.

[2] *The Physiology of Plants,* pp. 341 ff.

If now one place narrow strips of epidermis, or thin cross sections, from the middle of the hairy portions of these roots, in different proportions of potassium nitrate dissolved in distilled water—2, 2½, 3, 3½, 4, 4½%—one will find that, in the more concentrated solutions, the hairs collapse, or at least that the protoplasm contracts inward away from the walls. On the other hand, the hairs will swell somewhat in the most dilute solution, though this swelling is naturally hard to detect. In one of the solutions of medium concentration, generally in the 3% or 3½% solution, the protoplasm will withdraw slightly away from the walls. The solutions next lower in concentration will produce no visible effect; the one next higher will produce pronounced contraction. The solution of highest concentration which fails to produce any visible effect is said to be *isotonic* (p. 13) with the cell sap. The work of others[1] has shown that this solution will produce a pressure, as measured by manometer, of about ten atmospheres, or 150 pounds. In other words, water will enter the cell with a head or pressure or speed equal to the difference in concentration between the soil-solution, or the lake or stream water, or the sea water, and the cell sap. In ordinary soils, and under ordinary conditions, this is enough to supply land plants with the water which they require: but when the difference in concentration between cell sap and soil water falls, the pressure becomes insufficient, absorption becomes inadequate in speed or volume, the plant or part wilts.

Soil Water. Water is held in the soil by the chemical attraction between the water and the soluble constituents of the soil. It may also be held between the soil particles by capillarity. Water will move through or from the soil only in response to force greater than that which holds it. There must therefore be sufficient chemical attraction or osmotic gradient or other force or forces within the living cells, the root hairs, to overcome the water holding forces of the soil. No natural soil is ever absolutely dry in Nature; but between the available soil moisture, which can be absorbed and used by plants, and the total soil moisture, there is a remaining amount of water which may be geologically important but which is biologically useless. The aim of irrigation, in gardening and in arid regions, is to furnish available soil moisture in suitable amounts. It should not be excessive, but it should be sufficient. Different plants have very different water requirements. Cultivated as well as wild plants show these differences remarkably. Cacti, now favorites for amateur culture, require very little water, while most ferns need much. Oats require[1] more water than barley, which needs more than wheat, which needs one and one-half to twice as much water as millet and sorghum, to produce a crop.

---

[1] *The Physiology of Plants*, pp. 341 ff.

If then water is required by a plant with roots in the soil, and the soil water is so strongly held by capillary and chemical attractions that the osmotic gradient, if any, will not cause the water to move in sufficient rate and amount into the root hairs, absorption will be deficient, the plant will wilt. From this we see first, that the water *holding* capacity of different soils is different, and *second,* that while the water *requirements* of different sorts of plants are different, their water absorbing powers are not likely to be greatly different *unless* the density of the cell sap is greatly different. The differences among the plants of the same region are slight; but the differences between the characteristic plants of different regions are marked.[2] This can readily be shown by osmotic determinations with potassium nitrate solutions, as on page 13, of the concentration of the cell saps of salt marsh plants, swamp or bog plants, marine algae, fresh water algae, land plants of desert, stream bank, lake side, shade and sun plants, seasonal plants (wet and dry season plants), parasites and their hosts, or of whatever plants may be available.

---

[1] Briggs, L. J. and Shantz, *The Water Requirements of Plants.* Bulletins 284, 285, Bureau of Plant Industry, U. S. Dept. Agric. 1913. "Relative water requirements of plants," *Jour. Agric. Research,* 3, 1914; *The Physiology of Plants,* pp. 148 ff.

[2] *The Physiology of Plants,* pp. 37-9.

Cell sap determinations may, perhaps, be more conveniently made in mass than under the microscope. By the usual freezing point determination methods of the biochemical laboratory[1] we have another means of ascertaining sap concentrations. These, as is well known, are based upon the fact that solutes lower the freezing point of the water in which they are dissolved, the more concentrated the solution the lower the temperature at which it will freeze.

The matter of physiological interest is not the concentration or the diluteness of any given cell sap, but the ratio of its concentration to the concentration of the solution in which the plant or its root lives. Thus the very dilute cell sap of the plants of humid tropical jungles and the concentrated cell sap of marsh plants are both more concentrated solutions than the waters which they absorb. Absorption is possible and takes place; for, along the osmostic gradient from more water to less, water molecules will flow into the root hairs of land plants, and into the cells of water plants. Until and unless osmotic and electrical equilibrium is reached, water and dissolved substances will flow, through membranes permeable by them, into cells which, by living, prevent the attainment of osmotic and electrical equilibrium. A living cell, a living organism, may therefore be described as a self-maintaining osmotic system consisting of colloids, imbibed water, and dissolved substances.

Contact. One further consideration must be taken into account in dealing with absorption, for it is manifestly necessary that soil water and cell sap come into contact if absorption is to take place, and that the contact be sufficient in extent or area to ensure the absorption of enough water and dissolved substances. This is possible in such granular material as soil, in which the water is largely held on the surfaces of soil particles, only when the surface of the more or less bulky root is brought into direct contact with the water on these surfaces. This is accomplished when the epidermal cells, covering the cylindrical shaft of the root, grow out as hairs, slender enough to apply themselves to the irregular surfaces of the soil particles. We can readily demonstrate this by sprouting mustard or other small seeds in fine moist *black* sand in tumblers or even under the cover-glass under the microscope. The white roots, rootlets, and root hairs will be readily visible, the hairs clinging closely to particles of various shape and size but quite too small to be more than touched, at best, by the roots or even rootlets. Soil water, wet walls, wet cytoplasmic membranes, and cell sap constitute, therefore, a continuous mass of water throughout which movements of water molecules and dissolved substances inevitably take place, equally or unequally, in all directions, according to the prevailing pressures.

---

[1] C. A. Morrow, *Biochemical Laboratory Methods,* pp. 80-2.

CHAPTER III

THE MOVEMENT OF WATER

A. The Movement of Food Materials

Any damp or wet object will dry if exposed to the air. The land plants, with their stems, branches, leaves, flowers, and fruits in the air, are exposed to water loss by evaporation. This will be affected in amount and in rate by such surrounding conditions as the warmth, dryness, barometric pressure, and movements of the air, as well as by the structure and condition of the plants themselves. These various factors involve a knowledge of air and soil conditions which we may well refresh, as well as a knowledge of plants.

The Relations of Temperature, etc. to Evaporation. If, for example, we place equal amounts of distilled water in watch glasses of the same size and shape and put one on the table at room temperature, one on the radiator (if steam is on!) or other warm object, one in a cooler room, one in a draft, one in moist air, and one in a partial vacuum, we shall find them drying at very different rates, or, what is another way of stating the same thing, becoming dry in very different times. The results, however, are not satisfactory for by the terms of our experiment, we confuse the matter by altering more than one condition at once. Thus when we put the dish on a warm radiator, we subject it not only to heat but to air movement as well. If we put it into an oven, we unduly confine it and the surrounding air; but if we make all our experiments in the same or similar ovens, at different temperatures of the same air, in moist and dry air of the same temperature, in still and in rapidly moving air of the same temperature and the same humidity, and in the same air at ordinary and at reduced pressures, we shall so control our experiment that the results will be significant. Similar conditions necessarily affect plants. A hot dry wind, for example, takes water out of everything—the sea, the soil, foliage, flowers, fruit, as well as laundry hung on the line—much faster than a muggy day of the same temperature and no wind. Correspondingly, the vegetation of the Atlantic seaboard and of the Mississippi Valley is exposed to much less water depletion than the plants of the Intermountain Area, the deserts, and the Pacific Coast.

Waterproofing. If one put equal quantities of distilled water into watch glasses of the same size and shape, and pour melted paraffin upon one, oil of the same amount and temperature upon another, a piece of cloth over the third, leaving the fourth uncovered, but warmed to the same temperature as the others, and place them side by side in the same conditions of temperature, draft, etc., one will of course see that covering the surface of the water reduces the evaporation by a definite percentage per hour according to the imperviousness of the cover. Again, if one use two sheets of dentist's rubber, one sheet intact and the other with many small perforations, to cover two watch glasses of water similarly placed, one will see that the loss of water by evaporation through the holes is considerable, in spite of the imperviousness of the greater part of the perforated sheet of rubber.

We are now ready to apply the results of our observation of models to a study of plants. Cut off a leaf of garden nasturtium, coat the cut with a little vaseline, and weigh immediately. Similarly cut off, coat, and immediately weigh leaves of geranium, bean, apple, pine or other Conifer, fern, and as many others as one likes, choosing as great a variety as possible. Weigh each of two apples, of two potatoes, of two twigs with lenticels, peel one apple and one potato, and spot the *lenticels* of one twig with vaseline or melted paraffin or wax, and set all in one place, side by side on a table, the window sill, or on a shelf. Reweigh all daily, noting the effective waterproofing of the waxy peel of the apple and the corky coats of the potato and the twigs. Water loss, as well as gas exchange, is promoted by such openings through cork as the lenticels afford. The consequences, in both respects, can be gauged by the comparative losses of weight by the twigs with sealed and open lenticels respectively. As to the leaves, all lose weight, but also quite unequally, both in speed and in amount, as curves will show.

Lenticels and Stomata. Bark and the underlying tissues are provided, in the lenticels, with means of facilitating the exchange of gases between the inside of the plant and the outside world. The intercellular spaces in the lenticels remain continuously open until the lenticel as a whole is sealed by the formation of new tissue behind it. On the other hand, the leaves and the young parts of twigs, still growing in length, are provided with *stomata,* which generally open and close in response to stimulus of some sort.[1] These slits in otherwise water proof, but not gas proof, epidermis are provided with guard cells which, because they contain chlorophyll, are unlike the remainder of the epidermal cells and themselves manufacture sugar (p. 80). Thereby they control the concentration of the cell sap, the intake of water, and their volume and shape; for they open by day and close toward night, in consequence of water absorption following sugar manufacture.

One can form some idea of the opening and closing of stomata and its consequences by applying a strip of dry (blue) filter paper which has been dipped in a strong solution of cobalt nitrate, to the under side of a brightly lighted leaf, and another strip to one which has been in complete darkness for some time. The cobalt paper may be held in place against the leaf by strips of celluloid,[1] and the leaf should not be detached from the plant. The strip of paper against the lighted leaf will change color sooner, because its stomata are open, than the darkened one with closed stomata.

---

[1] *The Physiology of Plants,* pp. 139-42.

Leaf Structure. Study of leaves by microscopic examination of sections, made across the blade, and through it parallel with the surface, will show that the waterproofed, generally single-layered epidermis is interrupted at intervals by slit-like holes. These are continuous with the intercellular spaces throughout the tissues of the leaf. This can readily be shown in two ways: either by drawing air through the leaf into water, or by displacing the air by water. These can readily be done *first* by attaching the tube of a filter pump to a glass tube passing through one hole of the rubber stopper of a bottle of water into which the stalk of a leaf dips. The stalk of the leaf is held tightly in the other hole, the blade being in the air. Suction applied by the filter pump will cause bubbles of air to stream out of the submersed cut end of the leaf stalk. Or, *second,* one may submerse the blade, leaving the cut end erect in the air. In this case the air drawn in through the cut end will reappear as bubbles on the surface of the submersed leaf blade. (Are there more bubbles on one side of the leaf than the other?) A *third* experiment, consists in applying suction to a bottle, partly filled with water in which a leaf is wholly submersed. The bottle is closed with a one-hole rubber stopper. The suction will displace the air in the intercellular spaces of the leaf with water, making it increasingly translucent as it becomes increasingly water logged.

These experiments show that the living cells forming the blade of leaves border directly upon spaces, cavities, filled with air. Through the guarded openings of the stomata the air within the plant is continuous with the air outside; but just as the aqueous solution of cell sap may be different in composition or concentration or both from the aqueous solution of soil water, so the air inside a leaf my differ in composition from the air outside. There will, therefore, be gas exchange by diffusion through the stomata just as there is osmotic exchange through cell membranes. Whenever the air inside a plant is damper, contains more water vapor by evaporation from the cells bordering the intercellular spaces, the water will diffuse outward as vapor through the stomata into the dryer air outside. Thus the loss of water by evaporation into the intercellular spaces, and from them through open stomata into the air outside, is a process of molecular diffusion, through membranes from the cells into the intercellular spaces, and from one part of a continuous volume of air to other parts through the stomata. These purely physical processes of evaporation, osmosis, and diffusion are sometimes spoken of collectively as *transpiration.*

---

[1] An easy way to manage this is to fasten together by their ends two strips of thin celluloid, 1¼″ by 4″. This can readily be done by using acetone or one of the new "Duco" cements which harden very quickly. The two strips of celluloid can be sprung apart at any time to admit the leaf and the strip of cobalt paper, and the whole thing should be light enough not to weigh down the leaf unduly.

Mesophyll. Examination of a leaf from which upper and lower epidermis have been stripped or shaved, discloses the really very considerable total area of the chlorophyll-containing, food-manufacturing cells, which borders directly upon intercellular space, in other words is in direct contact with the air. The plant anatomists and the ecologists will point out the great differences in leaf structure, as well as in cell sap concentration (see p. 48), between plants of humid and of arid habitats.[1] Where the internal air of a plant differs but little in humidity from the air outside, the leaf will be correspondingly loose in structure; and *vice versa,* the leaf will be compact in structure and of less area where the internal air has substantially higher humidity than the air outside.

Stomata. The communication of the internal air of a plant with the outside air through stomata is also ample or scanty, according to the relation of internal and external humidity. Thus one finds many or few stomata, as shown by direct microscopic examination or by the cobalt paper test (p. 53), and the stomata are on both upper or lower sides of the leaf or on the lower side only. Experiment will show that such short lived annuals as horse bean (*Vicia faba*) will develop stomata in both upper and lower epidermis of the leaves, or only or mainly in the lower, according as the plants are grown in damp air and in a greenhouse, or in the open and in dry air.

---

[1] Haberlandt, G. (transl. by M. Drummond), *Physiological Plant Anatomy.* London, 1914.

We have thus seen that water enters the roots of land plants and passes off from the leaves, intake and loss being proportioned to the difference between the concentrations of the internal and external solutions, both liquid and gaseous. Thus cell sap and soil water in one ratio control water intake; internal air and the air outside in another ratio control water loss. While the water vapor in the intercellular spaces is the water naturally lost, this amount will obviously be large or small, will increase or remain fixed, according to the rate at which this water vapor can escape through open stomata or through the imperfectly waterproofed cuticular covering of the epidermis. When the water leaves the cell and becomes vapor, whether it reaches the air outside or remains within the intercellular spaces of the plant, it has ceased to be a part of the plant. Theoretically, for physical reasons, it may be reabsorbed; practically, for physical reasons also, it probably never is. Under ordinary conditions it escapes into the dryer air outside. Also, under ordinary conditions, it is only this passage of already lost water from the air within to the air without which is controlled by the stomata.

Hydathodes. When evaporation is slow and the internal atmosphere becomes saturated, the pressure of water within the cells may become too high for the holding power of the cell membranes. Then liquid water may be squeezed out or secreted by certain more or less specialized cells. These have been called *hydathodes,* and may be found at the ends of the veins on the round leaves of the garden nasturtium (*Tropæolum majus*), on the surface of the leaves of certain beans (*Phaseolus sp.*), and at the tips of blades of grass. In the last case the water squeezed out is often spoken of as dew. It occurs whenever water absorption by the roots exceeds water loss and the water holding power of the cells of the leaves or other aerial organs.

Guttation and Bleeding. The phenomenon just described is sometimes called *guttation,* but when the same exudation of water takes place through wounds it is called *bleeding.* Dew on the lawn can be imitated, and the various features of the phenomenon demonstrated, by sprouting grain on wet paper or cloth under a tumbler or bellglass in the laboratory, or in any other place where both air and substratum are very moist, as in a greenhouse, or in any moist chamber. The water, accumulating in drops near the tips of the blades of grass or grain, may be collected. If some of this is evaporated on a *clean* glass slide, it will be found to leave a slight deposit. This one should expect, for the cell membranes are permeable to dissolved substances as well as water. One may ascertain something of the composition of the extruded solution by various chemical tests. Of these the one most commonly successful is the Fehling or Benedict Solution test for sugar.[1]

Grape vines and other plants pruned or wounded in the spring are generally known to bleed or "weep." While weeping may be due to exactly the same physical phenomena of water intake in excess of water loss and water holding power, it may be accomplished by the aphids (plant lice) living upon a plant rather than by the plant itself. This may sometimes be observed under hawthorn or other shade trees planted at the edge of a sidewalk, upon which the aphids drop the sugary solution which they rob from the tree but cannot hold. Upon this solution, sometimes called "honey dew," molds may grow. If these produce dark colored spores, the result is a black sticky accumulation on foliage and sidewalk. This is "black honey dew."

---

[1] Fehling's Solution may be bought at a drugstore, but may easily be made. 5 gr. copper sulphate are dissolved in 20 cc distilled water in one bottle. 2 gr. sodium-potassium tartrate and 0.2 gr. sodium hydroxide are dissolved in 20 cc of distilled water in another bottle. A drop of each added to the concentrated "dew" and heated on the slide will yield the usual red copper oxide precipitate if a reducing sugar is present. Benedict's Solution is sometimes more convenient since only one liquid is required. Dissolve 17.3 gr. sodium citrate and 10 gr. dry sodium carbonate in 60 cc distilled water, warming to hasten solution. In 15 cc distilled water dissolve 1.73 gr. copper sulphate, and pour slowly into the sodium citrate-carbonate solution under stirring, dilute to 100 cc, and bottle. This solution will keep indefinitely, and may be treated like Fehling's, by warming after application to the material under examination.

Maple Syrup and Sugar. In this country in the spring, when the conditions above described are commonly produced, the native maple trees are made to bleed, and the very dilute but sugary outflowing cell sap is concentrated to the important commercial product, maple syrup. Still further evaporation yields the familiar brown maple sugar, with characteristic fragrance as well as sweet flavor. Maple syrup and sugar, therefore, are composed of the great variety of substances composing cell sap, namely sugar, water, salts, coloring, and other matters. "Pure maple syrup" or "pure maple sugar" exists only in the sense that the material comes entirely from the maple tree and not from the sugar cane, beet root, or other less expensive though wholesome source.[1] We can easily confirm some of these assertions by making a fairly dilute solution of maple syrup or sugar, filtering, examining, and identifying the solid accumulation on the filter paper; boiling the filtrate with bone black and again filtering, thus removing the coloring matters; treating a part of this filtrate with Fehling's or Benedict's Solution (see p. 19); and finally slowly evaporating the other portion and examining and identifying the crystalline and amorphous residue.

Various other peoples besides our own have learned to make plants bleed and to use the resulting exudate or flow. The Century Plant (*Agave*) is one of these, the expressed cell sap of which is fermented by the Mexicans into an alcoholic drink, *pulque*.

---

[1] *The Physiology of Plants,* pp. 168 ff., and literature there cited.

By bleeding, guttation, transpiration, and the synthesis of foods and fuels (pp. 74 ff.), the very considerable amounts of water taken in through the root hairs from the soil are lost and used in the upper parts of the higher land plants, in the foliage and in the less well waterproofed smaller branches. This is obvious, but can be proved by comparing surface and mass, "bark" and cork of a three inch length of twig, about an eighth of an inch in diameter, with the same features of a three inch length of branch, about an inch in diameter. The cut ends should be quickly sealed by melted paraffine. Twenty-four hours later weigh again. In proportion to the mass the small twig, with its greater surface and thinner "bark," has lost more water than the branch. A branch would lose more than the trunk: but through the roots, trunk, branches, and twigs water moves from root hairs to those cells on the intercellular spaces which lose water as vapor to the internal and external air (pp. 54-5). The course of this stream may be determined very exactly.

Conducting Tissues. If one cut off a white flower, a green leaf, or a short leafy stem, and place the cut end in red or green ink, one will presently see that the coloring matter goes up the shoot or leaf or flower stalk through the wood or the veins. The course of the vascular bundles in the white flowers is marked by the streams of color. Cross section or longitudinal stripping of the petal, will show, if promptly made, that the colored solution is in the wood or xylem portion of the vascular bundles. The position of the colored solution should be determined promptly because first, in case an ink is used, a poisonous preservative is also present, and second, many pigments diffuse more or less rapidly into the surrounding tissues, thus obscuring the picture. But if harmless colored solutions are used, one can produce, as florists sometimes do for special occasions, green carnations, red chrysanthemums, etc.

Acid Fuchsin and Light Green are two of the anilin dyes which may be used to mark the path of the *transpiration stream,* as it is sometimes called. To avoid the difficulty of diffusion very finely ground carbon in the form of India Ink or Chinese Ink has been used. The dry stick of ink is rubbed or ground in distilled or tap water. Carbon suspensions as one finds them ready made, in certain commercial inks, are not desirable for our purpose because of the presence in them of antiseptic, poisonous preservatives. Careful grinding in water with mortar and pestle will yield a fine carbon suspension. When the ends of cut stems are dipped into such suspensions, the carbon black is drawn up with the water. If there is direct communication from vessel to vessel (duct to duct), the carbon will be drawn along with the water; but if the vessels are closed at intervals, the carbon will accumulate in the closed upper ends of the ducts, which it enters. If a vessel is closed at both ends, as well as upon the sides, the carbon cannot enter. Also, if there are no vessels, as in the bulk of the wood of the Conifers, the carbon will enter only the cut ends of the tracheids and be drawn upward to their upper ends. This will be shown by longitudinal sections of the branches; but sectioning may easily so displace the uncohering granular carbon that its position under the microscope will be misleading.

The Course of the Transpiration Stream. The difficulties indicated above can be avoided by using starch instead of carbon, for this is readily converted into paste wherever it may be, and will stay in place. The procedure is as follows. Cut a stem, branch, leaf stalk, flower stalk, or fruit stalk *under water* in order to avoid breaking whatever continuity there may be in the threads or columns of water in the part to be experimented upon: and leave the material with the cut end under water until ready to transfer to the starch suspension.

Bearing in mind the small, often very small, diameters of ducts in the xylem portions of vascular bundles, and the various sizes of starch grains, one will wisely select the smallest grains available. Several starches are easily obtainable, of which Kingsford's Corn Starch, to be had in small packages in grocery stores, is finer than any I have been able to produce by small scale hand or power grinding. The fragments of the starch exploded in making Puffed Rice, Puffed Wheat, etc. are still smaller. If one dry Puffed Rice in an oven at such temperature that it does not brown, it becomes very brittle, and may readily be reduced to powder by mortar and pestle or by coffee mill. Put into water and allowed to stand for an hour or so, this material will separate, the heaviest part falling to the bottom, the cellulose fragments and other light stuff rising to the top, and an opalescent layer lying between. This is a starch suspension and may be siphoned off. Under the microscope the granules of starch are seen to vary greatly in size. To reduce the suspended grains, the suspensoid, to more uniform dimensions, one may centrifuge the opalescent liquid, again siphoning off the clearest supernatant part. This will be found to contain only very fine granules of starch, but that the granules are starch is shown by the fact that they and the liquid can be promptly colored blue by iodine solution.

Now transfer the amputated part from the water to the starch suspension thus prepared. The starch will go up the vascular system with the water. The experiment may be allowed to run as long as one please, for five minutes, until wilting, etc. Then the material should be dropped into boiling water or put into a steam sterilizer for a few minutes, to convert the starch into paste, wherever it is. The exposure to heat should not be so long as to make handling of the material difficult. It may then be stained by iodine solution, the starch turning blue wherever it is. Of course all the starch in the part, whether storage starch made by the plant itself, or suspended starch used in this experiment, will be stained by the iodine, and one must distinguish between the two. This is readily done by examining sections of a control part not subjected to starch treatment, and determining the position of the storage and other starch, if any. Storage and other starch does not occur in ducts or tracheids in untreated wood. Hence, whatever starch is found in the ducts and tracheids of the parts experimented upon is the starch deliberately introduced. Its position indicates the course of the transpiration stream up the stem to be through the ducts and tracheids of the vascular bundles.

If it is possible to amputate stem or stalk under the starch suspension, instead of first under water, and to begin observing at once, this is obviously most desirable, and should be done. Pines and other Conifers, herbaceous and woody plants and parts, slender climbers and sturdy trees, under conditions of high humidity and low, high temperature and low, insolation, cloudiness, and darkness, can all be tried. Not only the course but the rate of the transpiration stream can thus be determined as well as the lengths of bundles and ducts, the conditions in the bundles and among the ducts and tracheids and the course of the water from duct to duct and tracheid to tracheid. From the rate at which starch reaches a certain point in stem or stalk, and from the diameter of the vessels traversed, one may also compute the volume of water absorbed and moved during a given time and under the prevailing conditions.

The Course of the Sap Stream in Conifers. The wood of the conifers is composed largely of tracheids. These lifeless products of single living cells have so-called bordered pits in their radial walls. Cross and longitudinal sections of pine wood show that bordered pits consist of very thin parts of the wall protected on both sides by perforated dome-like or saucer-like structures. A pine twig cut off under starch suspension and allowed to stand in it a few minutes, steamed, stained with iodine solution, and then sectioned both across and also longitudinally, will show that the starch was stopped by the walls of the tracheids, but that it accumulated at certain points, namely at the ends of the tracheids, and also against the thin membranes under the domes of the tracheid from which the water moved. There is no starch on the other side; for whether there are or are not perforations through the membranes of the bordered pits, none are large enough in the conifers which I have tried to permit the passage of even such fine starch fragments as described above. But to conclude that, because the suspended starch does not pass through cell walls, neither does water is so manifestly false that the error is not made: for we know that water must pass or the parts above will wilt. The carriage of suspended starch into the cavities of cut tracheids shows that the course of the transpiration stream lies through them, just as, in stems with ducts or vessels in the wood, the carriage of suspended starch throughout the length of a continuous duct shows that the course of the transpiration stream is upward through them.

Xylem the Water Carrier. Since one finds suspended starch in the ducts and tracheids of the xylem, and not at all in the elements of the phloem, or bast, one must conclude that the classical idea that the course of the transpiration stream is upward through the wood is correct.

Sap Pressure. If one cut through a leaf stalk under starch suspension on a warm dry sunny summer day, and keep both cut ends under the surface for a time, then steaming, sectioning, staining, as before, one will find that the suspension has been drawn into both ends, upward above the cut toward the leaves, downward through the open cut toward the trunk or main stem of the plant. On the contrary in the spring when, in many other plants as well, the condition above described in the sugar maple exists, one will find that the starch suspension does not enter through either cut. Instead there may well be a certain amount of sap bleeding into the suspension. This observation, therefore, confirms what was said above as to the conditions underlying bleeding and guttation, and it indicates too that, in summer or at other times of rapid water loss, instead of the water in the ducts and tracheids being under pressure, it is under traction or stretching strain, *negative sap pressure*. The condition of pressure which may give rise to bleeding, prevails only when water intake exceeds water loss. This is when, in moderate temperatures, much water may enter the plant from moist soil while into moist cool air smaller amounts of water can be vaporized than into dryer warmer air. In the evergreens there may be the same surface at different times, but on the trees which put out a fresh crop of leaves each spring there is a rapid expansion of foliage. This, as indicated above (p. 54), results in the enormous increase in the cell surfaces bordering the intercellular spaces in the leaves. Into these spaces, through these surfaces, water is lost as vapor. Thus water loss may come to equal or exceed water intake. There results a condition either of balance or of so-called negative pressure, respectively.

From these observations it is evident that the water which goes up from roots to leaves is not *pushed up* at times of greatest need and of most rapid water movement. Nor is it *drawn up* by *capillary attraction;* for while capillarity develops very considerable force, it presupposes continuous tubes, and we have seen in the Conifers that the wood is made up of tracheids (p. 62). It is not *moved* by imbibition; for the water, once imbibed or absorbed by the cellulose of the cell walls, is *held* by it until a stronger force applied to the water molecules drags them away (evaporation). While conceding that pressure exists at times, and at such times pushes water upwards; that capillarity exists in capillaries in contact with water and therefore exists in the elements composing the wood of land plants, and tends to pull water and aqueous solutions upwards; and that imbibed water exists in the walls of ducts and tracheids; yet we cannot be satisfied that any or all of these bring about the varying, at times rapid, at times slow, movement of water from the absorbing roots to the transpiring leaves. Other factors must be involved. Probability indicates that the water is drawn or pulled up from above in response to a general condition, and that perhaps living cells are involved in the process. We can test both of these possibilities.

Tensile Strength of Water. If one touch the tip of one's finger to the surface of a drop of water, the two will stick together. If one gently raise the finger, they will continue to stick together. Until the finger has drawn the water up for some distance, the water molecules will cohere, the column will be maintained though becoming thinner and thinner. Finally, when the pull or tension exceeds the cohesive or tensile strength of the water, the water column will break into two pieces, the lower subsiding into the original drop, the upper rounding off upon the finger. This rounding off illustrates the surface tension of the water. By modifying this simple finger experiment one can gauge the tensile strength of water and of aqueous solutions more accurately. Dixon, in his studies of the ascent of water through the trunks of trees,[1] used experiments to determine the breaking strain of columns of water. This he found to be at least 150 atmospheres. Assuming the pressure of the air, one atmosphere, to be 15 pounds to the square inch, and capable of balancing a column of water 30 feet tall, a pressure of 150 atmospheres could sustain a weight of 2250 pounds to the square inch or a column of water 4500 feet, or nearly one mile, high. This is manifestly not realized in a tree or other living thing, for gravity, hydrostatic pressure, the strength of cell walls, and many other factors are involved. But we can at least conceive or imagine that water, because of its remarkable properties, including its great tensile strength, will be able, other things being equal, to hold together in any stem of any known height in spite of the suction exerted by the leaves.

The Suction Force of Leaves. If one seal the cut end of the petiole of any fairly large freshly cut leaf, cutting the leaf stalk under water, into a piece of barometer tubing filled with water above mercury, one will find the mercury drawn up into the tube as water is withdrawn from the tube to replace that lost by evaporation in the leaf. This experiment has been so successfully conducted, using a leafy branch instead of a single leaf, that mercury has been drawn up beyond barometric height. This indicates a suction force in excess of one atmosphere, i.e. of more than 15 pounds to the square inch, and it demonstrates that water has tensile strength equal to such evaporating or suction force as this.

---

[1] Dixon, H. H., *Transpiration and The Ascent of Sap.* London, 1911. "The tensile strength of water," *Proc.* Roy. Soc. Dublin, 12, 7, pp. 60-5, 1909.

It goes without saying that, when leaves and flowers wilt on an insufficiently watered plant, the evaporation from the leaves exceeds replacements through the vascular tissues. Whether this is due to a suction force greater than the tensile strength of water, to aqueducts too few or too small to allow enough water to pass in a brief unit of time, or to an inadequate absorbing system, cannot be asserted as a general principle. We may, however, make observations leading to conclusions on these matters.

If one grow some of the more vigorous varieties of sweet peas, for example the Spencers, out of doors against suitable support, watering them adequately but always along the soil and not by high sprinkling, one may have remarkably tall plants, say eight feet tall. The diameters of the stems, even at the base, will be between an eighth and a quarter inch. The root system, as shown by taking up sample plants, will be found to be extensive and apparently ample. The foliage will be moderate in extent and retain its freshness and greenness for weeks, *provided* that the humidity of the air does not fall too low and that there is no high drying wind. If one examine cross sections of the stems of the sample plants above referred to, one will find that the vascular system, corresponding with the slender stems, is also slender and that the ducts are small in diameter. In proportion to the foliage the vascular system is very long and slender. Its efficiency can be judged by determining the time in which starch suspension will be carried up a given distance in a freshly cut stem.

Vascular Efficiency. Comparison of various vines as to the area of foliage, of the cross section of vascular tissue, and of the root system, will give us a clue as to why sweet peas and certain other vines so definitely cease on the onset of the regular dry season. If one compare grape vine, morning glory, "old man" (*Echinocystis fabacea*), squash or pumpkin, with sweet pea, one will see the differences, and one will be led to conclusions as to the behavior of these different plants.

By means of color solutions and a horizontal microscope[1] one may time the ascent of sap in such translucent stems as *Impatiens sultani*, miner's lettuce (*Montia perfoliata*), etc. Cutting off the stem under the surface of the dye, and immediately bringing the top of the column of colored water into the field of vision, enables one to observe the swiftness with which the sap moves up the xylem of a vascular bundle. One can observe the same effect, if translucent stems or petioles are not available, by using white petals, white or variegated white and green leaves, or even ordinary green leaves and a harmless red solution (p. 60).

Are Living Cells Concerned in the Ascent of Sap? One may suspect that the remarkable differences in the behavior of these different plants under the same conditions, as of the same plants under different conditions, are due to differences in the behavior of living cells which may be involved in sap transfer. It is not easy to establish this suspicion or to prove that they are not concerned. The experiments of the advocates and of the objectors are almost equally faulty. Between the ingenious but pathological stabbing experiments of the Hindu Bose, and the poisoning or steaming experiments of the German Strasburger and the American Overton there is little to choose.

Bose's Experiment. The former connects a sensitive galvanometer with a probe which he inserts through the bark or epidermis into the stem to be experimented upon, in such position and to such depth that the probe reaches living tissue. The other pole of the galvanometer is set upon the outer uninjured surface of the plant. Of course a difference in potential is at once observable, and this difference is not constant. To infer, however, that such differences indicate the active participation of living cells in pumping water is to fall into an error in logic; for the effect that one sees and the cause that one sees are not at all necessarily coupled. In fact there is no evidence anatomical or physiological compelling one to believe that the living cells do mechanically suck or force water.

---

[1] Horizontal microscopes may be had of the Bausch & Lomb Optical Co., Rochester, N. Y., E. Leitz & Co., 60 East 10th Street, New York, etc.

The Strasburger-Overton Experiment. As in the copper sulphate experiments of Strasburger (*Physiology of Plants*, p. 138), so also in the steaming experiments of Overton, only those cells are put out of action which are reached by the killing agent. Thus, fit a split cork, at least 2 centimeters in diameter, neatly and tightly around a suitable stem or vertical branch. With quick drying "Duco" cement fasten the lower end of a stout paper cylinder 10 centimeters long to this cork. When this form is dry enough, fill it with melted wax or paraffin heated to over 100° C., and allow it to set. If the paraffin has carried a sufficient amount of heat to the medullary and other living cells between and in the vascular bundles, they will be killed, but how can one be certain of this? Comparison of the behavior of the foliage below and above this hot paraffin compress, and on adjacent undisturbed branches, will show by the wilting of the leaves if the water current has been affected.

But if the water current has been affected because enough heat has penetrated to the center of the stem to kill its living cells, the water as water, and not merely as part of a stream, has also been affected by the heat. Thus we know that water expands and contracts, varies in volume, as it absorbs or loses heat. It is, therefore, sometimes used as a thermometer. Being a volatile liquid, it passes over more and more readily and rapidly into the vapor condition with rise in temperature, and hence the pressure in the vascular system will change with the temperature. This may greatly alter the whole condition of water movement. Furthermore, its relations to dissolved substances change with the temperature, and oxygen or air which would remain in solution will escape, appearing as bubbles as the temperature rises. This phenomenon, perfectly familiar in the case of carbonated waters ("soda water," ginger ale, etc.), is no less true though less evident in such waters concealed from observation as those underground and in the vascular systems of land plants. If bubbles form under these conditions they will necessarily change the rate of water movement where they are formed and hence, throughout the system. The Overton experiment, therefore, demonstrates an effect but it does not reveal the cause as exclusively as the death of living cells concerned in water movement.

Summary. As the foregoing discussion and experiments show, the path taken by water and the substances dissolved in it—generally mineral salts, food materials—from the roots throughout the plant, to the leaves, is through the wood (xylem) of the vascular bundles. The aqueous solution is drawn upward by a suction force developed by evaporation which takes place from the living cells bordering the intercellular spaces in leaves and other parts exposed to the air. This suction force is directly proportioned to the rate of vaporization of water. It is dependent for its effectiveness upon the very high tensile strength of water. We can conceive, then, the phenomenon of the ascent of sap from roots to leaves as the drawing upward of *wires of water*. When this upward drawing is rapid enough to make good the loss of water due to evaporation, the plant and its parts will remain fresh and plump (turgid). When the movement is unequal to the need, the plant and its parts will wilt. Wilting may be due to inadequate absorption, or to inadequate vascular transfer of water to offset rapid loss. The evidence for and against the participation of living cells in sap ascent is equally inconclusive. We therefore conceive sap ascent as a physical phenomenon accomplished by the suction force developed by the vaporization of water, the tensile strength of which is sufficient to hold it together in capillary columns.

That these capillary columns, if broken, can be more or less perfectly reestablished is perfectly familiar; for we frequently revive wilted sprays of bloom or foliage by placing them in water, especially in a cool dark place. The advantages of coolness and of darkness, in this connection, are obvious, for evaporation is slower at low temperatures, and especially controlled by the stomata, which close in darkness (p. 53).

The revival of wilted parts is slow or rapid according to the speed of intake and transfer of water. The reestablishment of the capillary columns of water will vary in speed according to the shape, size, and distribution of vascular elements, as may be demonstrated by experiment.

B. The Movement of Foods

The transfer of the foods manufactured daily (see pp. 81-7) in the upper parts of the green land plant, throughout the body of the plant, also takes place only in solution in water, but the course of this movement cannot be as strikingly demonstrated as the movement of water and food materials. The relative volumes of the two streams determines this. The land plant moves large quantities of water only to lose it. The submersed aquatic plant moves very little water and loses none by evaporation. In the submersed aquatics vascular structures are said to be either undeveloped (as in the kelps) or to be merely the remnants of once useful and formerly developed vascular bundles (as in *Ceratophyllum*, etc.), when these plants are supposed to have lived on land. But both in land and in water plants of any but small size, the transfer of foods from the places of manufacture (pp. 96-9) to places of use or storage takes place in adequate structures. The phloem portion of the vascular bundles of the higher plants, and the sieve-tube-like structures in the kelps and even in rockweed (*Fucus*), furnish the main paths along which the dissolved foods move. As already indicated (pp. 57-8), dissolved food is in xylem as well as phloem, in ducts and tracheids as well as in sieve tubes, whenever the whole plant is gorged with water. The maple and similar trees in the spring illustrate this. Furthermore, where food is required in quantity to build large fruits as in pumpkin or to stock large seeds, as in buckeye, one may find sugar in the xylem of the adjacent bundles, though not throughout the vascular system of the whole plant.

If one, walking through a field of growing grain in the forenoon of a day of bright warm sunshine, pull and taste the stalks, one will be struck by the sweetness of the plants. Dextrous cross and longitudinal sectioning, and treatment with Fehling's or Benedict's Solution, with gentle warming on the slide, will demonstrate the presence of a reducing sugar. Examination under the microscope will show that the sugar is mainly in the phloem of the bundles. More skillful manipulation might prove it to be entirely in the phloem except as the sectioning had displaced it. This experiment can, of course, be duplicated, though probably with less striking results, on account of the weaker illumination, in a greenhouse or in a room.

Similar experiments, with other kinds of growing and actively food making and moving plants, will reveal the same condition. Furthermore, just as we have seen that starch suspension is not taken into the vascular system of plants filled with sap, like maple in the spring, so we can see that starch suspension is not taken into the phloem of a cut branch at any time. If the food is moved through the phloem mechanically, by pressure or by suction, one should be able to demonstrate this by cutting a stem or branch under the surface of starch suspension, keeping the cut ends of both stump and top under the suspension for a few minutes, and then treating and examining both as before. If suspension starch is found in the phloem of either stump or top, it is present only very near the cut. One reason for this may be the well known fact that sieve tubes very readily collapse when the ordinary tissue tensions are released by amputating, or even seriously cutting, a part. It is obvious that in the phloem there is a balance of forces, mechanical and other, no less delicate than that in the xylem. This balance is the more readily disturbed because of the relatively small volumes of the vascular elements of the phloem and of the food solutions to be moved.

Gravity may assist in the transfer of sugars, proteins, etc., from the leaves and other green parts where they are made; but it can do so, if at all, only where the place of manufacture is higher than the place of use or storage. There may be no gradient, as along a horizontal branch or stem, or the gradient may be adverse, as in erect fruits. Where food is required in quantity by large fruits or numerous or large seeds, the food may well reach these growing and therefore demanding parts along the same paths that carry the water with its dissolved raw materials.

CHAPTER IV

THE MANUFACTURE OF FOOD

I. The Raw Materials

All living organisms require food (*a*) to build and maintain (repair) their bodies and (*b*) as a source of energy to operate them. Crystals require a supply of the identical materials of which they consist in order to increase in size or in number, but the bodies of living things are not mere mosaics of elaborated foods nor machines burning their own parts to supply needed energy. Just as men build houses of wood, stone, brick, etc., but do not themselves consist of these materials, and just as men burn wood, coal, oil, etc. to supply themselves with warmth, steam-power, or electrical energy, so plants and animals build and energize their bodies with food, which is of different composition from themselves.

Food or *foods,* consist of comparatively complex chemical compounds formed from simpler substances. These raw materials are of almost universal occurrence in air and soil and, since the water washes the soil, in the natural waters of springs, streams, lakes, and oceans. What the raw materials and their sources are can be ascertained in two ways; *first,* by analyzing a sufficient number and variety of living organisms to reveal the materials common to them all; and *second,* by cultivating plants and animals supplied with these materials only. The first method requires the apparatus and technique of the chemical laboratory, the second involves skillful use of water culture and other artificial methods. By using these two means the possible errors of one procedure are corrected or eliminated by the other, and we are led to more nearly correct conclusions. That our conclusions from even extremely skillful and careful experiments are necessarily or invariably correct it is neither logical nor necessary to assume; and nothing confirms this cautious conviction better than the history of the subject of plant nutrition. We shall see this as we proceed.

The Composition of the Body. We have already seen (pp. 6-19) that a living cell, living protoplasm, and its products consist of proteins, lipoids, carbohydrates, and electrolytes. Analysis of these shows that they consist of compounds of carbon, hydrogen, oxygen, and nitrogen, of potassium, calcium, and magnesium, of sulphur, phosphorus, and iron. These ten elements are common to all living things; but sodium and chlorine are also always present; and aluminum and silicon are very common, for the one is characteristic of clay soils, the other of sandy, respectively. Certain animals contain and require a minimum of copper, ordinarily regarded as poisonous, and the highest animals have marked salt (NaCl) requirements. But if one start with seeds and cultivate the seedlings in solutions of air and water from which manganese and boron are entirely absent, the very small requirements of the plants for these two elements will not continue to be met by the stock provided in the seeds. This is a comparatively recent discovery,[1] for the classical statement is that only ten chemical elements are indispensable, the remainder being more or less in the nature of dietary accessories. Careful, detailed analysis of seeds of various sorts shows not only the main contents, which are indeed compounds of the ten elements named above, but also a great variety of compounds of other elements, the importance of which cannot be gauged at present. To minds prepared by the current talk about vitamins, the idea that more than ten elements are indispensable, although they may be present only in minute amounts, is entirely admissible.

---

[1] McHargue, F. "The rôle of manganese in plants," *Jour. Amer. Chem. Soc.*, 1922. Skinner, Brown, and Reid. "The effect of borax on the growth and yield of crops," U. S. Dept. Agric. *Bull.* 1126, 1923.

We know from every day experience that wood and other vegetable matters, and animal matters as well, may burn completely, yielding certain volatile or gaseous products, a certain amount of ashes or ash, and heat. The energy yield is very important and we shall return to it later (pp. 108-21). The ashy residue is more or less soluble in water and is readily amenable to chemical analysis. It consists mainly of the oxides of potassium (K), calcium (Ca), magnesium (Mg), sulphur, phosphorus, and iron; but over five times as many other elements have been shown to be present in plant ashes. The volatile or gaseous products of burning are easily demonstrated to be mainly water (H_2O), carbon dioxide (CO_2), and ammonia (NH_4).

Turning from these chemical elements to the compounds in which they exist in living organisms we find an extraordinary range in quantity within narrow limits of composition. The water content of living organisms and their parts is always high, ranging from 96% or more in watery fruits to 12% or less in lichens, seeds, etc. during periods of dryness. This may be ascertained, for example, by weighing a ripe tomato, apple, pear, orange, cucumber, melon, etc., and air-dry dormant seeds of pea, bean, sweet corn, field corn, the grains (wheat, oats, barley, rye), fresh grass or alfalfa, hay, etc., drying in an oven at low temperature to constant weight, and determining the percentage of water lost. The temperature should be raised only slowly in order to retain all the material except the water; but it may be held at 100° C., when the drying is nearly completed, until the weight is constant.

It should be noted that between dry and air-dry there is a material difference. This difference will be greater in humid than in arid regions for the same species. Part of this is the water of imbibition (p. 44), which is held very tightly and can be driven off only by the application of considerable force such as heat. It is the minimum indispensable to the maintenance of that structure which is called protoplasm.

Carbon. About fifty per cent of the dry weight of living things is carbon. It exists in the form of nitrogenous and non-nitrogenous compounds, complex in atomic structure and arrangement. Some of these we have learned, by the methods previously described (pp. 15-23) to recognize as carbohydrates (sugars, starch, cellulose) and proteins. There are many other compounds which can be studied by chemical methods, for which this may not be the place.

There are two principal sources of carbon as food material for plants: the carbon dioxide of the air, and hence also of water, and the carbonates and bicarbonates of soil and water. The latter have only recently been recognized as important sources, but physiological studies of "hard" and "soft" waters and of sea water demonstrate that many plants take in carbon otherwise than as CO_2 dissolved in water or as the weak carbonic acid. We can demonstrate this by cultivating a hard water *Spirogyra* or a *Ceratophyllum*, *Myriophyllum*, or *Elodea*, which has been kept in darkness long enough to have lost its starch, in a solution containing no other source of carbon than a duly small proportion of sodium bicarbonate. A fragment of any one of these plants introduced into such a sterilized culture solution, or into a sterilized natural hard water, in a flask connected with the air only through a U-tube containing a CO_2 absorber (NaOH, KOH, or similar agent), will make starch, other conditions also favoring. But if the sterilized solution be a natural soft water or contain no bicarbonate, the plant will make no starch, unless there is inward leakage of CO_2 into the apparatus. Various cultures should, therefore, be set up, in flasks or other thin walled bottles, in which hard water, soft water, and culture solutions with and without bicarbonates, all duly sterilized by boiling and barred from atmospheric CO_2. Control cultures not barred from atmospheric CO_2 must be made at the same time and under otherwise identical conditions. The plants, freed of starch by being kept for a time in the dark, should be put in small amounts in each flask (avoid overcrowding). The cultures may then be set on a window sill or in a greenhouse. Bubbles presently appear in the mass of *Spirogyra* filaments or on various parts of the larger aquatics used in the experiment, when the plants have been in bright light for a time, provided there is a source of carbon in the flask, either in the air or in the solution; but no bubbles will form in soft water barred from CO_2 or in a solution barred from CO_2 and containing no carbon, no matter how well lighted. Samples should now be removed from the plants from which bubbles had formed and from those that had not, the green chlorophyll should be extracted by hot alcohol, and the colorless material tested with iodine. The samples from cultures in which bubbles had been produced will turn more or less blue black, owing to the presence of starch; the others will only be more or less yellowish brown, from the proteins of the cells.

Similar water culture experiments may be made with such land plants as wheat, corn, etc., and the results will be similar. From the air the plants will absorb CO_2 if there is any, making starch in their green leaves. Plants grown in air lacking CO_2, but with their roots in hard water or in a solution containing bicarbonate, will produce starch under proper illumination. Plants from which their starch has been removed by standing in the dark for the necessary length of time, will or will not make more, therefore, when properly illuminated, according to whether they do or do not have CO_2 or bicarbonate as a source of carbon.

Experiments with potted plants are full of sources of error, for unless extreme pains are taken to exclude all carbon compounds from the material of the pots themselves as well as their contents and from the air, some starch will always be found in properly lighted plants. Perhaps it is worth while to demonstrate this by sealing potted plants under tubulated bell-jars barred from atmospheric CO_2. Such plants, under proper exposure, etc., will form less starch than similar ones sealed under belljars from which CO_2 is not excluded. Extracting the chlorophyll from the leaves by hot alcohol, and then testing with iodine, will reveal this.

From these experiments various conclusions can be drawn, namely: that CO_2 and bicarbonates are respectively the principal and the most important secondary sources of carbon for green plants; that green plants take these in and make starch; that this is done in the light only. The relations of temperature and various other factors to this process can similarly be worked out from these experiments or from similar ones so controlled as to apply to one or another definite question only. These we shall come to presently; but we may, for the present, limit ourselves to these three propositions and inquire further as to the course of the reactions taking place under them.

Photosynthesis and Warmth. What plant physiologists used to call the assimilation of carbon, now descriptively named photosynthesis, and constituting the most important synthetic process in Nature,[1] takes place daily whenever temperature, moisture, and illumination are suitable. By using *Elodea*, *Ceratophyllum*, or other suitable plants living submersed in fresh or salt water we can still further study this process. If small thrifty sprays of these plants be placed singly and upside down in test-tubes three-quarters full of the water in which they have been living, out of doors or inside, they will presently begin to bubble from the upper cut ends if set in the sunlight. Noting the temperatures of air and water, and counting the bubbles, we can relate temperature and bubbles; for we shall find that the number of bubbles per minute or the speed of the stream of bubbles, increases to a maximum at a certain temperature as it is raised, or decreases to a minimum as the temperature is lowered. We can warm the water by lamp or cool it by ice and reach definite conclusions. Reflecting on these observations, and on the many other similar ones which one may make under all sorts of conditions, in all parts of the world, we see as before (pp. 9-11) how closely related the photosynthetic as well as the other activities of living protoplasm are to temperature, and how the cold of winter and the warmth of summer control the production of food.

---

[1] *The Physiology of Plants*, pp. 42-55.

Photosynthesis and Light. If the same experiment is once more set up, and a favorable temperature maintained approximately constant, increasing the illumination by concentrating the rays of light by mirrors or lenses, and diminishing it by means of suitable screens, we shall find that the streams of bubbles vary accordingly; bubbles are formed more rapidly up to a certain maximum as the light is increased, and they are formed more slowly until they cease altogether as the light is diminished. It is interesting to note the influence of clouds or of cloudiness on photosynthesis, and to conclude that more food is formed on warm bright days than on cloudy ones, and in regions and seasons of abundant sunshine than in foggy ones.

Photosynthesis and the Supply of CO_2. Similar bubbling experiments may be made with soft water which has been boiled to remove CO_2, and subsequently quickly cooled without unnecessary shaking. No bubbling will occur, even from healthy looking plants, until the CO_2 has been restored, as it may easily be by exhaling (breathing outward) through a tube reaching to the bottom of the test tube.

The Effects of Certain Stimulants. Caffein, alcohol, ether, chloroform, cocaine, etc., added drop by drop to test tubes in which the production of bubbles by healthy plants in the light is well established, will manifestly affect the rate of photosynthesis as shown by the formation of bubbles. It will be found that the results of such experiments tally with those previously conducted (pp. 32-3) to demonstrate the visible reactions of protoplasm to these drugs.

The Gas Liberated. The volume and the composition of the gas liberated in photosynthesis can be determined by using any convenient device for collecting the bubbles as they escape from photosynthetically active submersed water plants. The top generally consists of a closed calibrated tube filled with the same water as below. The number of cubic centimeters of water displaced indicates the volume of gas liberated during a given time, at a given temperature, and at a given illumination. If the tube be now inverted and the gas tested by a glowing but not burning match, it will be seen that the match will promptly burst into flame. This leads one to suspect that it is mainly oxygen, and chemical test will prove it to be so. Quantitative methods will show that the volume of O_2 given off is approximately the volume of CO_2 absorbed, and we are justified, by this observation and various others, in at least suspecting that the oxygen comes from the CO_2. Since the reaction takes place in water, whether the cell is that of a land plant or of a submersed aquatic, it may be suggested by the equation

$$6\ CO_2 + 6\ H_2O = C_6\ H_{12}\ O_6 + 6\ O_2$$

This equation, while indicating two raw materials—CO_2 and H_2O—and two products—a sugar and oxygen—gives no clue to the process and stages by which this most important result is reached. Furthermore, it does not suggest the importance as previously indicated (p. 76) of other carbon compounds than CO_2 as sources of carbon, especially the carbonates and bicarbonates. Nor does the escape of the oxygen as visible bubbles imply that all of the oxygen produced in land and water plants immediately and directly escapes into the air and the water. Oxygen is always being used, directly or indirectly in respiration (p. 108); and some of it may well be that liberated in photosynthesis. Indeed, the oxygen freshly formed in photosynthesis has only too generally been regarded as a by-product, useful to other organisms but of no value to the plants liberating it. Very recent investigations by Cannon,[1] still in progress, indicate the consumption in respiration of this photosynthate, no less than sugar, in the deeper tissues and in the roots of land plants.

---

[1] Cannon, W. A. *Experimental Studies on Roots.* Carnegie Institution, Washington, D. C., Year book 24, 1925.

Photosynthesis. We already know that photosynthesis does not go on in darkness, but the varying quantities of light and the various components of sunlight or of artificial light may have significant effect on food manufacture. We can ascertain this with any degree of accuracy that we wish by experiment. If a cloud drift across the sun the effect on the bubbling of the water plants used in the previous experiments will be immediate. By determining the total quantity of energy in the sunlight at any given time and place, the amount absorbed by the glass and the water used in the experiment, and the amount passing out on the opposite side of the vessel, we can deduce the amount absorbed by the plant and calculate the quantity per unit of plant surface and mass. While such calculation will show that only a small fraction of the available light is absorbed and used in photosynthesis, the inference that photosynthesis is an inefficient process is quite the opposite of true. No process which produces all the food in the world, and does so without exhausting, or even seriously depleting the available energy, or forming great quantities of waste (e.g. saw dust, tailings, dust, smoke, etc.), or making noises, and without enormously expensive machinery, can be otherwise regarded than as remarkably efficient. It is slow, it is seasonal, it is dependent upon definite conditions of temperature, moisture and illumination, and upon unobstructed supplies of raw materials; but given warmth, water, and light, green plants growing in normal soil, air, and water will make food for themselves and their offspring, and produce a surplus available to the organisms unable to manufacture food for themselves.

It is hardly necessary to prove the obvious, for one knows that plants do not make food at night or in the dark; but one can show that only the green, chlorophyll containing, parts of leaves, and the leaves or parts of leaves which are lighted, carry on photosynthesis. Placing whole plants in darkness results not only in stopping photosynthesis, but induces other changes as well. If they are kept for some time in darkness, a whole series of changes occur. The later ones affect growth, and may well be considered under the heading of the formative influence of light (pp. 145-6). The earlier changes include the removal of starch, and other foods accumulated in the chlorophyll containing cells during the hours of daylight, and will be considered later (Translocation, pp. 70-2, 100-7). A moment's reflection will show that, if one is to test the effect of light on photosynthesis by its effect on starch formation, one must be sure of the amount of starch, if any, in the cells to be tested. Thus if one choose to experiment upon a healthy plant which has been in bright light, one must ascertain the amounts of starch in the parts of leaves to be used.

Only Chlorophyll Containing Cells Carry On Photosynthesis. To demonstrate this let us use, out of doors and undisturbed plants if possible, or at least well established potted plants. Thus veronica, oleanda, geranium, etc. with variegated green and white leaves, may have a sufficient amount of leaves tested with iodine to demonstrate the presence of starch in the green parts and none in the white parts. Continued exposure to bright light will be followed by the presence and perhaps the increase of starch in the green parts, but still none in the white parts. One can only infer, therefore, that gardeners' varieties of variegated plants are defective, to the extent of their white areas. On the other hand, red variegation or coloration does not impair food production, for the red parts contain starch as well as the green. Microscopic examination gives the explanation. The white areas of foliage consist of cells containing no chlorophyll: even the chromatophores are small and rudimentary. The red areas contain as many green chromatophores as the other cells: the red coloring matter is dissolved in the cell sap and merely masks the green. Why then do the white areas look white? The familiar phenomenon of total reflection from the small volumes of air in the intercellular spaces is the reason. This can very readily be demonstrated by displacing the air with water, by immersing a white and green leaf in water in a vessel, and exhausting it under an air pump.

The yellow shade or color of healthy or dying leaves and of ripening fruits is due to yellow pigments contained in the chromatophores. These may be masked by the usual green, or the proportions of green (chlorophyll) and yellow (carotin and xanthophyll) pigments may be such that the yellow predominate to a greater or lesser extent. The changes of color accompanying the ripening of fruits, and those taking place in autumn or earlier, are due apparently to changes in the coloring matter of the chlorophyll grains or green chromatophores in fruit and foliage. These changes may be induced in various ways, in Nature and in industry; as, for example, by insufficient rain, by coal gas and other poisonous vapors,[1] and by the so-called "processing" of lemons and other citrus fruits. In all of these cases either a green pigment is broken down into yellow ones; or, in a mixture of yellow and green compounds, the green are destroyed, leaving the yellow evident. As a matter of fact, no one knows which of these two chemical changes occurs; for no one can be sure that the pigments extracted from green leaves are identical with the pigments of the green chromatophores ("chlorophyll grains") of the living cell. There is indeed, in spite of the eminence of the chemists who have contributed most to the question,[2] ample reason to believe that they were working on and with derivatives and not the original substance or substances in the living cells.

Only Lighted Cells Make Food. Either the same plants above used or others may have strips of florist's or other "tin" foil laid across their leaves to exclude the light from certain areas. The experiment may be varied in several ways. If a sheet of tin foil, with a square, circular, star-shaped or other hole, is folded over a leaf so that the opening exposes a part of the green upper surface of the leaf, or if one use a glass or film negative with pronounced dark and light areas as a screen over a leaf, and the plant is exposed for several hours of daylight, there will be a decided and evident difference in the quantities of starch made in the unshaded and shaded portions. This can be shown both to the naked eye, and under the microscope, by iodine. First dip the whole leaf in boiling water, then place it in alcohol to extract the coloring matters, and finally lay the decolorized leaf in a white saucer containing the usual iodine solution in water ($I + KI +$ aq. dist.). In a few minutes the lighted portions of the leaf will have turned blue, blue-black, or black, whereas those parts under the tin foil or the dark portions of the negative will show little or no other reaction to iodine than the yellowing of their protein contents. Sections of the shaded and unshaded portions of the leaves examined under the microscope and treated with iodine will be found to contain exactly corresponding amounts of starch. In using these or other light screens one should observe certain precautions: the tin foil, or film, or plate should conform as closely as possible to the surface of the leaf, but it should not be clamped down upon it or fastened by vaseline or other adhesive, because thereby the entrance of CO_2 would be impeded and other gas and water relations interfered with.

---

[1] *The Physiology of Plants,* pp. 81-7.

[2] Willstätter and Stoll. *Untersuchungen über Chlorophyll.* Berlin, 1913. Jorgensen and Stiles, "Carbon assimilation," *New Phytologist,* 1915.

This becomes of economic importance where orchards border dusty roads, or are exposed to factory dusts; [1] for road dust, cement dust, soot, etc., forming an opaque coating over the leaves, cut down the photosynthetic activity of the foliage. This may seriously reduce the volume and quality of a marketable crop. While the greenhouse man may whitewash his glass to prevent overheating in summer, he takes pains to keep his glass as clean as practicable the rest of the year in order to shade the plants under cultivation as little as possible. In the short days of winter he may even find it profitable to supplement the sunlight by using powerful electric lights for several hours at night. To make this supplementary illumination of any real value, however, powerful lamps producing light of proper wave lengths must be used.

---

[1] *The Physiology of Plants,* pp. 81-3.

The Effective Rays. Daylight being composed of rays of many different wave lengths and chlorophyll evidently absorbing only some of these, one may well ask what are the rays of light furnishing the energy for photosynthesis. One may approach this question in two ways, *viz.* by examining the living and lighted leaf with the spectroscope, or by applying as light screens on living leaves the small thin gelatine films so colored as to yield only monochromatic lights.[1] Both methods are difficult, the former because of the many obstacles to be overcome in spectroscopic analysis,[2] the latter because one must use *equal amounts of energy*, whatever the wave lengths, before inference is justified by the results obtained. This is the stumbling block over which the classical and the modern experimenters have tripped. If one can secure small gelatine plates of red, orange, yellow, etc. colors, which pass equal quantities of energy though of different wave lengths, one may justly conclude that certain wave lengths are more effective than others. At the present moment we do not actually know, and the values of various illuminants, glasses, etc. are open to question. It seems probable, however, that the rays of longer wave lengths, nearer the red end of the spectrum, are more useful in food manufacture, and that the rays of shorter wave lengths, at and beyond the violet end, are more useful otherwise,[3] as we shall see later (pp. 145-50).

---

[1] The so-called Rattan Filters, obtainable from the optical companies.

[2] Mestre, H. "The investigation of the pigments of the living photosynthetic cell." Contributions to Marine Biology, Stanford University, 1930.

[3] *The Physiology of Plants,* pp. 72, 318.

II. The Foods Made in Photosynthesis

In the foregoing experiments the production of starch has been demonstrated, but we must regard this substance not as a first product but as a form in which some chlorophyll containing cells temporarily store the food they have made, whereas other chlorophyll containing cells similarly store oil, fat, or sugars. More permanent storage of foods takes place elsewhere and will be discussed later (pp. 95-9). As previously indicated (p. 80), we know the raw materials and the finished products of photosynthesis, but we do not know the intermediate stages or the intermediate substances.[1] Presumably carbon dioxide and water are combined to form a simple sugar which is raised to a more complex one, finally a hexose sugar, glucose. Various sugars simpler than glucose have been found in green leaves. These are claimed to be the early products of photosynthesis; but so are formic acid and formic aldehyd, although the evidence is by no means convincing. Not only is photosynthesis the most important chemical process in Nature, but it is also one of the most difficult to understand.

To demonstrate that other substances than starch are produced by photosynthesis we can use green leaves of various sorts and certain common fresh water algae. Thus, remove a green leaf of an onion plant growing in sunlight, dry it to brittleness in an oven, at a temperature sufficiently low not to burn or even brown the leaf, and grind to powder by pestle and mortar. Divide the powder into two very unequal parts. Test the smaller portion with Iodine Solution, demonstrating that starch is not present. Extract the larger portion with about twenty times its volume of distilled water and presently filter. Divide the filtrate into two parts. To one part add Fehling's or Benedict's Solution at once and warm. To the other part add a few drops of hydrochloric acid and warm for some time, then testing with Fehling's or Benedict's Solution.

---

[1] Spoehr, H. *Photosynthesis.* Chemical Catalogue Co., New York, 1926.

The precipitate coming down in the part of the solution to which the test liquid was immediately added, indicates the amount of *reducing* sugar in the aqueous extract, whereas the larger amount in the portion first warmed with HCl shows more nearly the *total* sugars present, because the HCl inverts cane and other sugars which will not reduce the copper salt of the Fehling or Benedict Solution. If starch were present in the leaf and suspended in the water extract, it too would be converted into sugar by digestion with HCl in warmth.

In the fresh or salt water alga *Vaucheria*, and in diatoms, one can observe that oil drops occur in proximity to the chromatophores, just as starch grains do in other cells. That the drops so observed under the microscope are really oil drops can be proved, either by extracting them with such oil solvents as ether, choloform, etc., or by staining them with such characteristic oil stains as Tincture of Alcana Root. Osmic Acid, often recommended as an oil test, may be quite misleading, for it stains tannin and various other things as well as oil, and therefore fails to distinguish oil from the others.

III. The Other Foods

The Immediate Products of Photosynthesis are thus proved to be sugars, reducing or other, in solution in the cell sap. The sugars may be (1) temporarily stored as such in the chlorophyll containing cells in which they are formed, or (2) they may be temporarily stored as granules of starch, or (3) they may accumulate as drops of oil, or (4) they may be removed at once to other parts of the plant. But these various substances consist only of carbon, hydrogen, and oxygen. They are not complete foods for protoplasm, which consists of compounds containing nitrogen, sulphur, and phosphorus. These must be added to the sugars. One should therefore ascertain whatever is possible about the nitrogenous compounds of carbon; but it is obviously very much more difficult to distinguish on the one hand between the nitrogenous constituents of protoplasm and its nitrogenous products than, on the other hand, between protoplasm and the non-nitrogenous products of photosynthesis. In fact the syntheses of nitrogenous foods which can directly nourish protoplasm are not understood. The energy used, the place of manufacture, the sources of nitrogen, the course of the reactions, the intermediate and final products, are all subjects of study and of speculation. We know that soil nitrates, sulphates, and phosphates are required and used by plants; we know that nitrogen, sulphur, and phosphorus containing and correspondingly complex compounds of carbon are made and used by plants, and required and used by animals; we know that amino acids occur in plant cells, increasing and diminishing in quantity and varying in kinds, according to circumstances; but details of the actual syntheses of complex nitrogenous foods still remain for the future to disclose.[1] Methods of supplementing the nitrogenous food materials and foods of plants are used by fungi, both saprophytic and parasitic, by other saprophytic and parasitic plants ranging in development from bacteria to dodder, by the carnivorous plants, etc.; but experiment upon them may be deferred to more special occasions.[2]

We can, however, demonstrate that nitrates are the chief source of nitrogen, that sulphates and phosphates are the sources of sulphur and phosphorus respectively. This is done by employing what are known as water cultures. These are so easy to use in a rough way that one is prone to forget that, to obtain reliable results, one must take extreme pains to introduce into the culture medium only the compounds desired. One has to take into account the glass or other material of the culture vessels, not only to have it chemically clean, but to determine what ions, if any, will be taken up from it by the water. Most glass is more or less soluble, and potassium and other ions go off into the culture solution, affecting its reaction (pH) as well as its other relations to the organisms in culture. The water must not only be distilled, but it must be much purer than commercial or usual distilled water. The "conductivity water" of the physical laboratory is none too pure for exact work. The chemicals must be pure and uncontaminated in use. Finally, when every other precaution has been taken, one must deal with the plants themselves. They contain in their seeds, roots, etc., as we already know (pp. 73-5), a great variety of compounds of many different elements. These will suffice for a time to meet the requirements of the plants; they may even offset unfavorable proportions in the nutrient solution itself. Regulating humidity, temperature, illumination, maintaining the suitability of the nutrient solution, and keeping it from infections, constitute the exacting requirements of those who wish to obtain reliable results from water culture. Just as we saw (pp. 76-8) that neglecting to determine whether a hard water or soft were to be used in photosynthetic experiments on aquatics vitiated our results; so one may omit the obvious source of nitrogen, namely nitrate, from the culture solution, but the ammonia fumes of the laboratory and many other nitrogen compounds, will suffice to obscure purely qualitative results whatever quantitative determinations may show.

---

[1] *The Physiology of Plants*, p. 96.

[2] *Ibid.*, pp. 98-119.

Water Cultures. Let one use Mason jars, culture solutions consisting of weighed chemicals and commercial distilled water, grasses and leguminous plants started in all sorts of places, and one will come out with more or less satisfactory *demonstrations;* but these must not be regarded as *proofs.* Thus we make a solution consisting of 1.0 gr. $Ca(NO_3)_2$, 0.25 gr. KCl, 0.25 gr. $MgSO_4$, 0.25 gr. KH_2PO_4 in 1000 cc distilled water, to which a drop or two of some soluble Fe compound must be added. An identical solution, containing the same ions except NO_3, should be made, using 1.0 gr. $CaSO_4$

instead of the calcium nitrate. The culture vessels, solutions, stoppers, etc. must now be thoroughly sterilized, and clean corn seedlings with roots about 1 cm. long may now be installed in the culture bottles. There are various ways of doing this, all more or less undesirable! Corks with holes of suitable size to hold the seedlings: the corks must be sterilized and then dipped in melted paraffine. Wire screening, with mesh of proper size to allow the roots to go through, without permitting the seeds to drop through; must be galvanized, *not* coppered, and should also be dipped in paraffine. Mosquito netting, of suitable mesh, and paraffined. The neatest way, perhaps, is to have rings of glass-tubing, over which mosquito netting may be stretched loosely and fastened by thread or by paraffine. The nutrient solution should cover the roots of the seedlings without more than touching the lower part of the seeds; but the seeds should be protected from undue drying until the cultures are well established. In ten days or longer the seedlings deprived of nitrate ions in the culture solution will be lagging in growth behind the others, but one should not ignore the possibility of an unfavorable effect of the $CaSO_4$ as well as the NO_3 poverty.

One may wonder, realizing the extent to which ammonia occurs in soil and air, owing to the decay of organic wastes, etc., whether ammonia compounds may not suffice as sources of nitrogen for green plants: but when one reflects that the ammonia may first undergo change to nitrate, the value of the question is lessened. Yet one may make a third solution, and a third simultaneous set of cultures, the nutrient solution containing 0.50 gr. ammonium phosphate and 0.50 gr. calcium sulphate in place of the 1 gr. $Ca(NO_3)_2$ of the first solution. This set of cultures should theoretically be intermediate in growth between the two previously described.

Deficiencies, disproportions among the soil constituents, unfavorable physical properties, and perhaps other qualities of cultivated soils may be corrected, as man learned by experience ages ago, by the use of manures and other fertilizers. The reasons for the value of fertilizer practice are evident from the preceding experiments and others of similar nature. But one may also realize that soils, fertilizers, and culture solutions may be of such sort or amount as to overstimulate the plant to undue vegetative growth and deficient flower, fruit, or seed production;[1] though here again, the nutrient solution, whether in natural or cultivated soil, or in artificial culture, offers only one set of factors in a complex environment which also includes light, warmth, and water as constituent factors.

IV. The Ash Constituents

While experience has taught man that he and his fellow living beings, plants and animals, consist in part of substances which remain as ash after burning, science has not yet revealed the specific rôles, if they have them, of the various elements contained in the ashy residues. The most abundant and constant of these elements are Calcium, Potassium, Magnesium, Sulphur, Phosphorus, and Iron; but Silicon, Aluminum, Manganese and even Copper and Zinc, appear in the ashes of certain plants or in certain regions. The last two are the more noteworthy because they are strikingly poisonous to plants. This property is used by man in protecting himself or his cultivated plants by poisoning undesired or parasitic competitors with salts of copper. Thus copper sulphate is commonly used in reservoirs, swimming pools, etc., to check the growth of algae; and it is an important constituent of sprays and dusts used in orchards, gardens, etc., to control fungi (Bordeaux mixture, etc.).

Small amounts of many elements are found in seeds and spores. These are present in the form of suitable, useable compounds, and in amounts sufficient to supply the early needs of seedlings and sporelings. This can be shown by sprouting seeds of mustard, radish, etc. in good distilled water on filter paper; under suitable conditions of warmth and light. They will grow well for a time, but before the seedlings need the mechanical support usually afforded by the growth of the roots into the soil, it will be noticed that the leaves are not normal in color, perhaps not in size and form. If one attempt to determine which ones of the many chemical elements are most needed, one can gather ideas by modifying this experiment in various ways. Germinate suitable seeds, as above, on filter paper kept moist with distilled water, and when the seedlings are of convenient size, pot some, and place others in solutions made as follows:

---

[1] *The Physiology of Plants*, p. 97.

1. in distilled water
2. in distilled water with a small fragment of calcium nitrate
3. *ditto* with a small fragment of potassium phosphate
4. *ditto* of magnesium sulphate
5. *ditto* with calcium nitrate and magnesium sulphate
6. *ditto* with calcium and potassium salts
7. *ditto* with potassium and magnesium salts
8. *ditto* with all three
9. *ditto* with all three and a few drops of ferric chloride

From these rough and purely qualitative experiments, suitable only for brief runs, one may pass to the carefully planned and skillfully executed water cultures previously discussed. Nevertheless, these rough little ventures suggest certain ideas deserving reflection. The salts used break, in the suitable dilutions above used, into their component ions, the base and the acid carrying positive and negative charges respectively. In ordinary natural waters, such as the soil solutions, the streams, lakes, and the sea, the ions of acid and base, and of calcium, potassium, and magnesium, neutralize each other, offsetting what has been called their antagonisms, and maintaining a reaction (pH) approximately neutral. On the other hand, there are many waters occurring in Nature or employed in the arts, of which this is by no means true. Either the idea of balanced solutions is groundless, or our notions of what constitute the limits of life are quite too restricted. Some, not to say many, organisms can live under "fatal" conditions. On the other hand, experience has taught man that some soils are "good," others "poor" or "bad." These qualities are due to unsuitable proportions of the different elements, to be corrected by suitable fertilizers which make up for deficiencies or remove excessive quantities of one or another soil ingredient. The province of soil fertilizers is one upon which we cannot now enter; but it may be pointed out that fertilizers may accomplish either or both of two things *first*, improving the composition of the soil, and *second*, changing its physical condition so that its components are increasingly useful. Chemical fertilizers can well accomplish the first; but they affect the latter very little. Stable manure is an admirable fertilizer, because it contributes to both beneficial results. Humus, leaf mold, and loam are also desirable additions to certain soils because of their physical properties as well as their chemical. They hold water admirably, thereby preventing or delaying excessive drying, and also diminishing the speed with which rains, etc., drain through sandy soils. Their chemical value, on the other hand, lies in their organic rather than in their mineral content.

It may further be remarked that plants, like animals, require somewhat different dietaries at different ages. The proportions of the mineral salts, as well as of water,[1] are not uniform throughout the lifetime even of the annuals. It has already been pointed out (p. 74) that seeds and spores contain the amounts and proportions of the raw materials, foods, and fuels necessary to successful germination and early growth, and that the solutions subsequently used must supply the larger amounts required for later growth. Similar changes in the soil solution itself no doubt regularly occur in Nature, as they may very well be brought about[2] in cultivation and in cul-

[1] Bakhuyzen, H. L. van de Sande. "Physiological phenomena at the time of flowering," *Proc. Soc. Exp. Biol. & Med.*, 24, 1926.

[2] Gericke, W. F. "Salt requirements of wheat at different growth phases," *Bot. Gazette*, 80, 1925.

tures. The normal progress of the seasons, especially in those regions where there are regularly alternating rainy and dry seasons, brings about changes in the concentration, and in the composition as well, of the soil solution which cannot fail to produce their effects in living organisms, especially in the longer lived annuals. This is strikingly illustrated by the "miner's lettuce" (*Montia*) and the hayfield tarweeds (*Hemizonia*) of the Pacific Coast.[1] With the onset of the dry season, when the rains cease, the soil dries, the atmospheric humidity falls and the day temperatures rise, miner's lettuce dies. Then the rosettes of broad somewhat succulent leaves of tarweed begin to send up from the drying soil slender dry woody stalks bearing few and widely spaced small dry leaves. Concentration, composition, and even reaction of the soil solution range between the minima of midwinter and the maxima of early fall. This seasonal annual change is illustrated also by the waters and by the flora and fauna of ponds,[2] streams, and even of the lakes and the coastal portions of the oceans.

---

[1] *The Physiology of Plants*, p. 335.

[2] Scott, F. M. "Introduction to the Limnology of Searsville Lake." Stanford University Publication. *Biological Sciences*, Volume 5, No. 1, 1927.

CHAPTER V

THE STORAGE OF FOODS

In the foregoing pages the experimental evidence has been described which shows how living things obtain the water, gases, salts, and finished foods and fuels, which they need. Experiments have been suggested from which one can draw conclusions, or theories, as to how raw materials enter living bodies, and are made over into finished foods and fuels, by the application of energy to the raw materials. These finished products may be used at once in metabolism (see the following chapter), or they may be used at once in growth and maintenance (see Chapter VIII), or they may be stored for future use. The forms, or compounds, in which foods and fuels are stored, and the places of storage, in plants, are of great economic as well as scientific importance, for mankind lives largely on food stored in plants. The places of storage—fruits, seeds, stems, roots, leaves—are more familiar than the composition of the foods; and this is much more familiar than the ways in which the foods come to the places of storage and are packed there. While the first question is one of structure and morphology, and the second of organic chemistry, the third concerns the physiologist primarily. Agriculture and economics are as largely unconscious of the value and importance of plant physiology to them as many botanists are of the opportunities and obligations afforded them.

Foods in Transit. The immediate results of photosynthesis are the accumulation, in the actively food manufacturing cells, of non-nitrogenous foods in solution (sugars) or in solid granules (starches). As previously shown (pp. 81-2), the chlorophyll containing cells in green leaves will accumulate sugars and starches during the daylight hours, whenever photosynthesis exceeds the rate of removal. By shading or darkening a leaf or part of a leaf we can so cut down photosynthesis that there will be no temporary surplus (p. 79). We can reduce photosynthesis by lowering or raising the temperature past the optimum, by excessive or insufficient water supply, and by other means. But while there may be no temporary surplus there may still be photosynthesis, the production of sugars, and the removal of these from the leaves to other parts of the plant for use or storage. This can be demonstrated by applying Benedict's Solution to the longitudinally split leaf stalk or stem of the plant under examination, and gently warming. If a reducing sugar is in transit, the characteristic precipitate will form.

Also one may be able to demonstrate that nitrogenous foods are in transit. If one make two thin longitudinal *slices* (not thin *sections*) of the petiole or of the inner bark of the plant under experiment, laying them on a clean glass slide uncovered, and allowing them to dry spontaneously (i.e. without warming or heating), one may secure crystals of asparagin or other amino acids, visible in the phloem cells under the microscope. In the form of sugars the non-nitrogenous foods (starches, oils) move; in the form of amino acids the nitrogenous foods (proteins, etc.) move. The movements may be molecular (osmotic), from cell to cell, or they may be mass, as in ducts, tracheids, sieve tubes, etc. Wherever these foods occur in sufficient amounts, their presence can be demonstrated, with sufficient care and skill.

Storage, and subsequent digestion, follow photosynthesis and the further elaboration of foods. Temporary storage in green leaves (as above, and on pp. 86-7) is followed by digestion and removal of the starch. This is due to the action of amylase, diastase, an enzym which converts the insoluble starch into soluble malt sugar, maltose. This action can be demonstrated by applying an opaque covering to part of a green leaf and noting that, instead of starch accumulating it decreases, and presently disappears, in the covered green cells. Meantime a reducing sugar may be found in the vascular tissues of the shaded parts, as Benedict's Solution, gently warmed, will show.

In such leaves as those of onion, starch is not accumulated. Sugar will be found in them in increasing proportions as the day goes on. Experiments may be made to determine whether the sugar is a reducing one or not, and whether both reducing and non-reducing sugars are present.

Although the photosynthate in onion leaves is sugar, the non-nitrogenous food may be stored as starch or as oil in the leaves of the bulb. In the sugar beet the photosynthate temporarily laid down in the leaves as starch is accumulated and stored in the roots as cane sugar, although housewives claim to be able to distinguish between sugar from sugar cane and from sugar beet. In dahlia and Jerusalem artichoke temporary accumulation of photosynthate in the chlorophyll-containing cells is in the form of starch, but in the tubers underground non-nitrogenous food is stored as inulin, a soluble isomer of starch. This can be demonstrated very easily. Drop pieces of dahlia tuber into strong alcohol or concentrated glycerine and leave for several days. On examining thin sections under the microscope one will find sphaero-crystals of inulin in the parenchyma cells. The centers of these sphaero-crystals are very commonly on or in the cell walls.

In the nuts of ivory palm, used for button manufacture, reserve cellulose, an insoluble isomer of starch, is laid down in remarkable abundance. This can be shown either by *digesting with hot cuprammonia,* which dissolves cellulose, or by treating thin sections under the microscope with the usual aqueous iodine solution (I + KI + aq.) followed by sulphuric acid (see p. 4), which will turn the enormously thickened cell walls blue.

The non-nitrogenous foods may be stored as oil, in nuts, and in various other places. For example, if one grind the kernels of yellow field corn in a coffee mill, soak this in ether or chloroform shaking thoroughly, for a few minutes, and evaporate, one will recover a quantity of pleasant smelling red brown oil. On the other hand, if one subject a sweet corn, yellow or other, to the same treatment, one will recover a very much smaller amount of oil, if any. Similarly one may extract oil from leaves, and from certain algae, and diatoms. One must distinguish, as chemists do, between fixed and volatile or ethereal oils. The latter contribute the flavors and fragrances so highly prized in wild and cultivated plants. They may constitute reserves of food, exert a protective (repellant) or attractive influence, or they may be merely the useless by-products of vital processes. One can readily think of examples of each category. The fixed oils, or fats, on the contrary, are extremely useful foods or fuels. In both of these latter cases, however, they must be digested before they can be used. This is accomplished by enzymes called lipases (see p. 104 in the next chapter).

The nitrogenous foods must also be stored, to ensure the most complete preparation for the future. We find this to be the case. For example, in sections of mature potato one will see cubical crystals in the cells which, instead of turning blue or black, on the application of iodine, turn yellow or brown, thus indicating their protein nature. Similar material will be found, on the application of corresponding tests to other storage cells in stems, roots, and seeds. The demonstration of protein reserves in oily seeds sometimes requires especial care. If, for example, one extracts the oil from pieces of castor bean, and then sections the endosperm and embedded embryo one will find, on staining with the usual iodine solution, or Millon's reagent,[1] or Raspail's reagent,[2] that there is abundance of reserve protein in crystalline form in the endosperm cells, and ample amorphous protein in the protoplasm of the embryo. We have here, therefore, in one section, indeed in the same cells, demonstration of the nitrogenous reserve food from which the organized protoplasm may supply itself for maintenance and for growth.

---

[1] Dissolve one part by weight of mercury in two parts by weight of concentrated nitric acid, warming if necessary; dilute with an equal volume of distilled water, allow to stand, and use the clear supernatant liquid.

[2] Concentrated sugar solution followed by concentrated sulphuric acid, should produce rose red or purplish coloring of the proteins.

CHAPTER VI

DIGESTION

Since we know from foregoing experiments (pp. 34-6) that solid particles cannot pass from cell to cell, except as they may first be converted into the state of finest possible division—that is, dissolved—we must consider the means by which solution of the solid constituents of plants may be brought about. In the preceding chapters we have seen that the usual chemicals, alkalis and acids, exert characteristic effects upon cells and cell contents; but these reagents do not occur in living cells in the concentration employed in flasks and test tubes in the chemical laboratory. Therefore we must seek, in the living cells themselves, other agents which accomplish those chemical changes which convert substances that do not dissolve in water at ordinary body temperatures into others that do. These changes, known as digestion, are accomplished by and in the living cells themselves. While they may best be shown in germinating seeds, it is perhaps as well to try some of them in glass before turning to the vastly more complicated condition within living cells. For quantitative work one should make use of the methods of biochemistry; [1] but for general enlightenment, before resort to such methods, one may make the following tests.

If one scrape the white cut surface of a potato and place a small amount of the material upon each of five glass slides, and examine under the microscope, one will see the familiar, large, clamshell shaped, grains of potato starch. There may be broken grains as the result of the mechanical operation of scraping; but otherwise the grains will be intact. Now add a little water to the white scraping, cover with a cover glass and place a *very* small amount of dry diastase at the edge of the drop of water on one of the slides. Put a small amount of saliva upon the starch on another slide, and cover with a cover glass. Add a little water to the starch on a third slide and cover and then put a minute fragment of a moist yeast cake at the edge of the cover glass. For a fourth slide prepare a leaf extract as follows: dry a dozen green leaves in an oven at a temperature not exceeding 50° C. When they have become brittle, grind them in a mortar to fine powder, and then add twice the volume of water, shake frequently, and after two or three hours filter. Add enough of this clear filtrate to the starch on a fourth slide to fill out the cover glass. To the starch on a fifth slide add only water and a cover glass over the starch. Place the five slides where they will be warm *but will not dry out,* i.e. they should be kept in a warm moist chamber. This can be accomplished so readily and in so many different ways that it is enough to suggest this to the ingenious reader. If one examine the starch grains on these five slides twenty-four hours later, one will discover that the grains in all but one show more or less corrosion, and still later the corrosion will be even more marked. If any corrosion shows on the slide to which one added only water, one will also discover the presence of rod shaped bacteria. From these bacteria and from the yeast there diffused into the water some agent which corroded the starch grains by dissolving (converting, digesting) the starch. The leaf extract and the saliva contain similar material, which also acts on the starch, converting it into sugar which dissolves. The diastase is such a purified and concentrated extract made, however, from germinating barley.

---

[1] Morrow, C. A., *Biochemical Laboratory Methods.* New York, 1927. Gortner, R. A., *Outlines of Biochemistry.* New York, 1929.

Now one may turn to germinating seed, and also repeat on a larger scale these slide experiments, thus: soak some good barley over night and then place it between sheets of moist filter paper[1] under glass and set in a warm place for a day or two. When the kernels have sprouted and the radicles are about 0.5 cm. long, one may dry the germinated grains, again at temperatures not exceeding 50° C. If one open one of the drying sprouted grains, scraping off some of the material inside the kernel, and examine this under the microscope, one will find that many of the grains of starch are markedly corroded. One can detect grains in all stages of digestion. Comparison with similar scrapings of ungerminated barley will show that in the dormant grains almost no corrosion has occurred. When the rest of the material has become quite dry, the grains should be run through a clean coffee mill and ground as fine as possible. This material should then be soaked for two hours, with frequent stirring, and filtered off. The filtrate may be treated in either of two ways: *either* it may be concentrated by evaporating off more or less of the water; *or* it may be treated at once with strong alcohol (96%). In the latter case a whitish precipitate will be formed, which may be collected by filtration. This may be dried on the filter paper in an oven of low temperature, say 50° C. Also the concentration indicated in the first method must be accomplished at the same low temperature. If the concentrated yellowish liquid is now cleared by filtering, and the filtrate treated with strong alcohol, the same whitish precipitate will be formed. This can be filtered off and gently dried on the paper. If now this precipitate, which may otherwise be kept indefinitely, is redissolved in water, it will be found to act on the slide under the microscope and in a starch suspension or paste in the test tube, in exactly the same way as the commercial diastase of the preceding experiments. In fact it contains diastase.

Diastase, or amylase, the significant ingredient of malt extract, as experiments similar to the preceding will show, is a digestive agent, an enzym, which acts on starch in such a way that the starch molecule ($C_6H_{10}O_5$) unites with a molecule of water, and goes into solution as a sugar ($C_6H_{12}O_6$). Diastase is one of the many hydrolyzing enzyms. Enzyms are physiological catalists or catalysers. Definitions and descriptions of catalysis vary; but the enzyms may, in general, be conceived as substances which increase or decrease the rate at which reactions would proceed, under the same conditions, without them. The statement is sometimes made that no chemical change in the cells and bodies of plants and animals takes place without enzym action. While this statement has by no means been completely proved, the idea is acceptable because many of the reactions occurring in cells would not proceed at the rates they do unless the temperature were much higher than that of living things.

---

[1] If one prefer, for economy's sake, to use paper towelling, one should be sure that the paper towelling is as neutral in reaction as filter paper!

It may be interesting to show that the products of starch digestion in the above cases differ from such a familiar sugar as that stored in cane and beet. If one test ordinary commercial sugar (sucrose) with Benedict's or Fehling's Solution, one will get no copper oxide precipitate; but if one test the product of starch digestion, whether in bread or otherwise, one will demonstrate the presence of a "reducing" sugar, maltose. The "reducing" sugars are freely soluble, and also are more readily acted upon by the "respiratory" enzyms (see p. 121) than is cane sugar, which must be "inverted." Thus we see yeast added to bread dough, digesting the starch and converting or inverting the cane sugar, sucrose, to glucose, which it thereupon oxidizes (or ferments, see pp. 117-9) to alcohol and CO_2.

A study of the various enzyms acting digestively upon the various carbohydrates of plants—the starches, celluloses, sugars, and their derivatives such as the pectins—may be regarded as in the province of biochemistry or physiological chemistry, and reference is here made to guides in this field; [1]

---

[1] Morrow, C. A., *Biochemical Laboratory Methods*. New York, 1927. Gortner, R. A., *Outlines of Biochemistry*. New York, 1929.

but one may well illustrate here the digestion of other insoluble foods than the starches and celluloses.

The fats and oils are derivatives of the sugars formed in photosynthesis. They form the other most important group of non-nitrogenous stored foods. In the germination of fatty and oily seeds (castor bean and nuts), even in the germination of field or fodder corn (as contrasted with sweet and pop corn), these immovable and stable foods must be digested. They are acted upon by enzyms known as lipases, which convert the fat or oil into glycerine and fatty acids. These undergo oxidation in the respiratory process. Indeed, while castor bean seeds in the resting condition will scarcely yield a color reaction when an aqueous extract is treated with Benedict's or Fehling's Solution, they will show an abundance of reducing sugar after germination has been going on for several days. There will, of course, be a corresponding decrease in the amount of oil in the seeds. The extraction of the lipase from the germinating seeds is by means similar to those described above for obtaining diastase.

The other great group of stored foods includes the proteins. These nitrogenous organic compounds are amenable to the action of suitable enzyms. The enzyms acting upon the nitrogenous carbon compounds, like lipases, amylases, etc., are known by their effects rather than chemically; for although it is possible to secure some of them in quantity, as for example diastase, it is not yet possible to secure them in such purity that the determination of their composition is possible. Hence, as before, we may experiment with commercial products, such as pepsin, and with germinating seeds. Such experiments, if repeated with due care and refinement will lead to quantitative results such as the biochemical laboratory should furnish.

If one sprout lupin, barley, and similar seeds one will find not only that the starch diminishes in quantity, but that the protein reserves do also. The process seems to be, in general, hydrolysis, that is, an uptake of water by the protein. New proteins are formed, more soluble and less readily coagulating. Further changes result in the appearance of albumoses, peptones, and amino acids in the cells.

These changes can be effected in test tubes in the following ways. Cut the white of a hard boiled egg into small, clean, sharp-edged cubes and place one in each of six test tubes; cut six cubes of fresh lean beef and place one in each of six other test tubes. Notice the firmness of the beef and the sharp edges of the egg cubes at this stage, the beginning, of the experiment. Press out and filter the juice of a *ripe* pineapple. Put 5 cc of the undiluted juice into two of the test tubes of egg and two of the test tubes of meat. Dilute this with 5 cc of 0.25% hydrochloric acid in one of the tubes of egg and one of meat, diluting the pineapple juice in the other two tubes with 5 cc of distilled water. Similarly put 5 cc of a solution of Papoid (a commercial preparation from *Carica papaya,* containing *papain*) into each of two tubes of egg and two tubes of meat, diluting the Papoid solution with 5 cc of 0.25% HCL in an egg tube and a meat tube, and with 5 cc of distilled water in the other two tubes. Similarly with 5 cc of a solution of commercial pepsin in two tubes of egg and of meat, diluted with 0.25% HCl and with distilled water respectively. To these six tubes of egg and of meat should be added four more tubes, two containing cubes of eggs and two containing cubes of meat, two of which should be covered with 5-10 cc of distilled water and of 0.25% HCl respectively. The sixteen tubes should be cotton plugged and incubated at 35° C. for six hours or at a lower temperature if more time should elapse before examination. The liquid in each test tube should be tested with blue litmus paper just before cotton plugging and the acidity or neutrality of each solution recorded.

After the lapse of the necessary time, scrutinize the tubes containing the coagulated proteins of egg white and the uncoagulated proteins of beef. Are any of the edges of the cubes of egg impaired? Which ones? Is the solution in all flasks as clear as at the beginning, has the acidity changed, and what does the microscope disclose as to the nature of the liquid in each test tube? Is there evidence of bacterial as well as enzym action? Are these changes more marked in acid or neutral solution? Is the meat equally firm in all the tubes, and is its present firmness equal to its firmness at the beginning of the experiment?

One may now test the liquids for products of the digestion of egg and meat. These may be, as above indicated, proteins, albumoses, amino acids, etc. Among the common color reactions used to prove the presence of proteins are the so-called biuret and xanthoproteic tests, and the Millon reagent used earlier (p. 99). The first of these may be applied thus: to one of the solutions add a few drops of strong KOH solution and shake thoroughly; add a drop or two of 3% $CuSO_4$ solution and shake again. A clear violet color will ensue if proteins are present. The xanthoproteic test consists in boiling the suspected solution with strong nitric acid, whereupon if proteins are present a yellow and later an orange color result. The third or Millon's test consists in putting a few drops of Millon's reagent [1] into the suspected solution and heating in a boiling water bath. The resulting precipitate gradually turns to a pink and rose red color and may disappear, leaving a clear red liquid.

The foregoing tests, applied to solutions sufficiently incubated, will indicate the production of soluble proteins from the egg white and the meat by the enzyms employed. In the case of the test tubes containing only distilled water or dilute HCl (0.25%), the enzyms effecting the production of soluble proteins are produced by bacteria, carried into the water by the egg or the meat, or already present on the glass or in the water. The different amounts of the protein products of digestion would be indicated in the different tubes if the same test were applied to an equal volume of solution in each. From such tests one may conclude whether these digestive enzyms are checked or favored in operation by the acidity of the medium (solution) in which they work. The slight acidity of the cell sap, the slight alkalinity of the protoplasm, as we have previously seen it (p. 25), obviously affects the digestive processes taking place in various parts of the animal and plant body. Germinating seeds, opening buds, growing fruits, demand and use so much food per unit of time that digestion of stored foods, nitrogenous and non-nitrogenous, is indispensable. It is accomplished by enzyms.

---

[1] For formula see p. 99.

CHAPTER VII

OXIDATIVE METABOLISM

All organisms require both matter and energy—matter for the formation of their body-substances, energy to do "work." In the foregoing experiments we have studied the means by which plants absorb their food-materials, elaborate these into foods, transfer or store and digest them, and build them into their bodies. One source of energy we have studied—the light; why is this needed at all? Is not this source of energy sufficient? It does not appear to be; for every living thing breathes, and by this means *liberates* energy. Energy is not *generated* by respiration; it is only liberated. Where then does it come from?

From the start we should bear in mind that the characteristic product in respiration is *energy*—much of it liberated in the form of *heat*. Respiration does not consist in an exchange of gases, it is not a means of ventilating or aerating the organism, it does not consist in an intake of oxygen and an output of carbon dioxide. Oxygen is ordinarily taken in and carbon dioxide is ordinarily given out, but this is not necessarily the case. There are, for example, certain bacteria which oxidize iron compounds, and certain others which oxidize ammonia compounds or other nitrogenous substances, giving off the corresponding oxides. Again, we shall see that carbon dioxide may be given off even under conditions which preclude the intake of any oxygen; and, on the other hand, it may not be given off when the intake of oxygen is unhampered. The one constant feature is the liberation of heat.

It has always been impossible to show that the heat liberated in respiration equals the amount of energy which the substances involved would yield in similar circumstances if completely oxidized. In other words, the energy liberated is not all in the form of heat. It is liberated in other forms, some of it is converted into work, the residue becoming evident as heat. We may demonstrate these various possibilities by experiment. The usual course, perhaps because it has always been the easier, is to determine and measure the material products of respiration rather than the yield in energy: yet the physician knows that the temperature of the human body is a much more significant indicator of his patient's condition than the rate or the products of his respiration. There is, in fact, a "normal" temperature for peas as well as for human beings; and any departure from this "normal" must be interpreted accordingly. Thus if wheat or other grain, or seeds, in storage or in transit, develop a higher temperature than normal, it may be because they contain too much moisture. They should be dry before putting into containers, or into cars or holds for transport.[1] On the other hand, the high temperature may be due to contamination or infection by fungi or by bacteria. These may cause "heating" of grain or of hay. Or the grain, or hay, especially if old, may remain abnormally cool. This will be found to be due to debility and is quite generally incurable in all organisms.

. . We may follow these general statements by demonstrations of various sorts. In the first place it may be remarked that those insulators devised by Sir James Dewar, as containers of liquid air, solid carbon-dioxide, etc., and now in common use under the names of Thermos or Icyhot bottles, have displaced other devices for insulating small volumes of air and of perishables. The principle is that heat waves cannot traverse a vacuum and may therefore be kept within or without a space enclosed by a vacuum. Dewar glassware is double walled. The walls are silver plated, as are mirrors, and reflect heat and other rays as mirrors do. The space between the inner and outer walls is as completely exhausted of air as possible and sealed. A Dewar flask, therefore, or a thermos "refill," is a silver plated vacuum.

---

[1] *The Physiology of Plants,* pp. 194-8.

We may begin our experimental work with a quickly sprouting early variety of table peas such as American Wonder, of the preceding harvest.[1] Suppose one weigh out three lots, of 80 grams each, of peas as they come from box, bottle, or sac. One set (#1) should be placed in a 250 cc Dewar flask or half-pint thermos refill, with 80 cc of tap water. A thermometer should be inserted and the neck of the flask closed with absorbent cotton. The thermometer should be read at intervals, and at the end of twelve hours whatever water has not been absorbed by the peas should be poured off. Another set (#2) of peas should be soaked in tap water for twelve hours, and then planted in clean sand. The third set (#3) should be dried in an oven at low temperature (75-80° C.) until the weight is constant. This dry weight subtracted from the original weight of 80 grams indicates the amount of water in air-dry peas, and from it one can calculate the percentage of water. Air-dry seeds contain 10-12% of water. If the moisture content be higher, the seeds are unlikely to "keep" well. This is shown, with serious results sometimes, by grain in storage or in transit. The drying of all digestible, putrescible, and living materials, as a means of preserving them, must be carried very far, and the dryness must be carefully maintained, if the desired end is to be reached. Thus dried beef and dried fish will keep perfectly if kept dry enough; and nothing will happen to seeds, spores, etc., as long as they are dry enough.

Meantime we should be noting that the temperature is steadily rising in the flask of peas. If the temperature is read for several days, it will be found to reach a maximum, and then gradually to fall. Futhermore, if the room in which the experiment is made has a considerable range in temperature, the temperature inside the flask will be correspondingly affected, though always with a lag, the minima and maxima inside the flask following the minima and maxima in the room. The lag will be greater and the fluctuation less the better the quality of the flask. The percentage of germination of #2 should be determined. How many days after planting does the first germination take place? On what day do most of the seeds sprout? When is the last germination? And what are the growths of root, shoot, seedling, at the end of one week, two weeks, and three?

---

[1] If one have older peas of previous harvests, other peas of other varieties, comparisons of their activity, vitality, germinating power, etc., will be interesting.

On opening the Dewar flask, the #1 set of seeds will be seen to be more or less badly molded. The question at once suggests itself, how much of the heat liberated, retained in the Dewar flask, and indicated by the thermometer, is released by the peas and how much by the molds and bacteria? To answer this question one may either attempt to sterilize the seeds, or to employ some agent to deter the growth of the fungi and bacteria. Various sterilizing agents may be tried, especially those in common use. Suppose one make several aqueous solutions: one containing 1% carbolic acid, one a 1 per M corrosive sublimate solution, one a 1% copper sulphate solution, one a 1% formalin solution. The Dewar flasks, thermometers, etc., as well as the seeds, should be sterilized; they should all be washed in sterilized water until all residue of the antiseptic is removed; and the mouths of the flasks should be promptly closed with sterile cotton plugs. Into a fifth, and a sixth flask, similarly sterilized together with its thermometer, etc., one may put an equal weight of soaked unsterilized seeds, and about one-tenth as much powdered sulphur, into one of these two flasks and plug as before. In due time one will notice that the temperatures developing in the different flasks are unequal, and on opening the flasks after four days one sees that the germinations are unequal, being least in the most perfectly sterilized seeds, best in the unsterilized seeds mixed with sulphur. The temperatures run strikingly parallel with these observations. It is, in fact, very difficult to sterilize the surface of seeds, either resting or sprouting, without hurting the embryos within. This hurting shows itself in failure to sprout, in diminished percentage in sprouting, or in smaller poorer seedlings. On the other hand, the presence of powdered sulphur serves only to check the growth of the invaders, the bacteria to a certain extent, and especially the fungi; but it by no means injures the embryo or other parts of the seeds. It is what is called an aseptic, not an antiseptic. It is unfavorable to foreign growths, but is not fatal to them. It is a very valuable constituent of antiseptic, insecticidal sprays and dusts. Its effect on the surface of a leaf in daylight, and especially in warm sunlight, and its effect in a Dewar flask, where light is lacking, are very different. In warm sunlight presumably more or less oxidized poisonous sulphur compounds are formed. Pentathionic acid is said to be one of them, and is a powerful fungicide. In the darkness of a Dewar flask it will not form until the temperature rises. Then, presumably, it is formed spontaneously in the warmth and dampness accompanying the germinating seeds, and it acts accordingly upon the small freely permeable cells of the bacteria and fungi.

By the use of thermometers in the preceding experiments we have demonstrated that energy in the form of heat is liberated by germinating seeds, but we have not indicated the quantity of energy, nor have we shown that man and other organisms are like peas and other quickly germinating seeds in releasing energy in the form of heat. The latter we can do at leisure. Using any suitable organism, whenever convenient, we can domonstrate that heat is given off by the organism, by exhalation or by radiation or by perspiration. We can show that, other things being equal, the amounts of heat given off correspond with the activities of the organism, the more active the organism, the more energy liberated. Although the thermometer indicates the presence of a by-product, heat, instead of the whole product of energy, it nevertheless indicates that energy and activity stand in relation to each other as cause and effect. This is shown, for instance, by the differences in the temperatures in similar Dewar flasks containing (1) equal weights of dormant and germinating peas, beans, etc.; (2) equal weights of slowly sprouting and quickly sprouting seeds such as pines and peas; and (3) corresponding weights of salamanders and mice. The list may be indefinitely extended, but it indicates that the slow going cold blooded animals liberate much less energy per unit of time than the warm blooded, that slow growers and quick growers correspond, and that sleep (dormancy) and activity require and accompany unequal supplies of energy.

How large or small these supplies of energy may be one may ascertain by determining the quantities of heat liberated. This can be done roughly in rather simple fashion, but accurate results involve so many precautions that we are justified in not attempting them here. The rough method is this. If fifty grams of a quickly sprouting variety of peas, which have soaked for twenty-four hours in water are placed in a Dewar flask, duly plugged, and kept in a warm place at even temperature, they will raise the temperature, as the thermometer will show. If now fifty cubic centimeters of cool, sterile, distilled water of known temperature be quickly poured into the flask, shaken for a moment by whirling movement, and the temperature read, one will know something about the *quantity* of heat liberated by the peas. The temperatures should continue to be read and noted, minute by minute, until there is no difference in the readings. Since a certain *amount* of heat is required to raise the temperature of anything, the rise in temperature of the definite volume of water indicates the amount of heat available. Physicists have established as a measure of heat the amount required to raise one cubic centimeter of pure water one degree centigrade in temperature.

They call this unit a calorie, or small calorie. The quantity of heat required to raise the temperature of 1000 cc or 1 Kilo of distilled water 1° C. in temperature is known as a Great Calorie or a Calorie, the same word as before but with the initial letter capitalized. If the temperature of the 50 cc of water is raised one degree, then they have absorbed 50 calories. These 50 calories have come from the peas. But the difficulties begin at once; for one realizes that, not only has the added water taken up heat, but all the other things—thermometer, flask, cotton, and even the moist peas themselves—have taken up quantities of heat. The fifty grams of moist peas have kept themselves warm and have raised the temperature of the whole system. They have liberated not merely 50 calories, but the numbers of calories required to raise the temperature of the different objects and to maintain this temperature in spite of losses by radiation, leakage, etc. The quantities of energy in the form of heat which germinating peas, and other seeds, liberate is therefore very considerable. But again this does not represent the total amount of energy liberated in the oxidative metabolism (respiration) of these living things; it is only the amount of unused, and unuseable, energy. The rest of the energy liberated has been used, converted into work of the many sorts involved in living. Hence we may infer that the total amount of energy liberated in a living organism or a living cell, and available for work, equals the amount liberated in the oxidation of the materials "used up" in germination, and other vital processes.

The quantity of energy released in the complete combustion of a given weight of wood is equal to the amount of energy liberated in converting the wood to charcoal plus the amount liberated in burning the charcoal. In similar fashion living organisms carry on their metabolic processes in stages. Some organisms never complete the series of oxidations, never obtain the maximum possible yield of energy from the material consumed. We may make this evident in two ways, namely by observing the heat yields and by determining the products.

If one weigh out two sets of 50 grams each of air dry, quickly sprouting peas (I use "American Wonder extra early" but other varieties are also excellent), and two sets of 50 grams each of hemp seeds *of the last preceding harvest,* and place one set of each in separate Dewar flasks, with 50 cc of water on the peas and about 25 grams of wet filter paper under the hemp seeds, one will have the means of determining the heat yields of these two kinds of seeds. The other set of each may be placed in separate bottles closed with ground glass or rubber stoppers. The Dewar flasks and bottles should be kept together and in as uniform temperature conditions as possible. After twenty-four hours, one may open the bottle of peas and gently decant the gas above the peas into a test tube half full of filtered barium hydroxide solution, and shake the test tube. The solution should at once become milky. Similar testing of the gas above the hemp seeds will produce little or no whitening of the solution.

The explanation may be found by other and less crude tests for carbon dioxide and barium carbonate. In the first place, if the milky liquid in the first test tube is treated with a few drops of HCl and clears up after effervescence, the inference is justified, in the circumstances, that barium carbonate was the white precipitate. If the milkiness persisted after adding HCl, one would suspect the presence of barium sulphate. In the second place, one may connect the two bottles with an aspirator so that a measured volume of the gas above the peas and above the hemp would be drawn through barium hydroxide solutions of known concentration. But one must be sure that the air passing over the peas and the hemp contains no CO_2. This is accomplished by passing the air first over or through a concentrated potassium hydroxide solution and then through a barium hydroxide solution before it is drawn into the bottles containing the seeds. The concentrated KOH solution absorbs the atmospheric CO_2, as is indicated by the continued clearness of the BaO_2H_2 solution.

If, after a suitable length of time, the precipitate in the barium hydroxide solution connected with the peas is filtered off, dried, and weighed, one can calculate the amount of CO_2 produced by the peas.[1] Similar treatment of the precipitate, if any, in the barium hydroxide solution connected with the hemp will indicate the great difference in the behavior of the two sets of seeds.

Obviously both peas and hemp, unless sterilized for this experiment, will harbor bacteria or fungi which completely oxidize carbon compounds as sources of energy. There should, therefore, be a small production of CO_2 in the hemp bottle, unless precautions were taken to prevent. What the products of hemp respiration may be one can ascertain by chemical analysis,[2] whereupon one will see that they are the products of the partial oxidation of foods (non-nitrogenous mainly) stored in the seeds. They are intermediates, their formation being accompanied by the liberation of some energy in the form of heat and otherwise; and these intermediates, when further oxidized, yield still more energy.

In animals nitrogenous compounds of carbon, as well as the fats and oils and the carbohydrates, are used in oxidative metabolism and yield energy. The oxidations are generally incomplete and the intermediates are excreted as urea, etc. In plants which manufacture their own food, orthotropic or independent plants, the nitrogenous organic compounds are much less involved in oxidative metabolism, the carbohydrates, fats, and oils furnishing most of the energy liberated. Hence, where ammonia, urea, etc. are of vegetable origin, they are generally found to be the products of the activities of bacteria or fungi saprophytic or parasitic upon plants or plant remains. Their activities are sometimes called fermentations, decay, decomposition, putrefaction, disease, according to man's attitude toward them. In them all, nitrogenous or non-nitrogenous compounds of carbon produced by other organisms, and either cast off (excreted) by them or constituting parts of their bodies, are acted upon, broken down to simpler compounds with corresponding release of energy used by the organisms accomplishing the fermentation, decay, or disease. We can illustrate these statements by experiment.

---

[1] The atomic weight of barium being 136.4, of carbon 12, and of oxygen 16, the amount of CO_2 in $BaCO_3$ would be 22.4% of the whole weight.

[2] Gortner, *op. cit.* Morrow, *op. cit.*

The fermentations[1] with which we are most familiar[2] are those accomplished by yeasts, either the beer yeasts, which are also employed in bread making and domestic doctoring, or the wine yeasts. These plants should be studied under the microscope if not already familiar. We may begin our experimenting by rubbing up a piece of moist yeast cake—say a cubical piece about a quarter inch on each face—in 100 cc of 5% sugar solution. If done in a mortar the rubbing should not be too hard or rough lest the breakage of yeast cells be excessive. Fermentation tubes may be filled with this suspension and set aside for the accumulation of the gas produced. More of the suspension may be poured into a flask or bottle which connects by means of bent glass tubing with another bottle containing freshly filtered barium hydroxide solution under which the glass tube dips. Still more of the suspension may be used in an absorption train for the quantitative determination of the CO_2 produced as previously described (p. 115). The precipitate presently formed in the barium hydroxide solution can be determined, as described on page 115, to be barium carbonate. This is formed by the oxidation of the sugar, water and carbon dioxide resulting as in ordinary respiration.

While carbon dioxide is the product most desired in the fermentation by which bread dough is "raised," alcohol and certain aromatic compounds are the ones for which beer and wine are produced. The production of alcohol in yeast fermentation can be very readily indicated by the following ingenious test. If the precipitate, formed in the barium hydroxide solution above described, is filtered off, and the filtrate gently warmed with a few flakes of iodine and a little potassium hydroxide solution, there should be a distinct odor of iodoform and on cooling, a yellowish precipitate. Quantitative determinations of the alcohol, etc. may be made by the methods of the biochemical laboratory. It will thereupon be found that, as in all other vital processes, the phenomena vary in rate, quantity, and quality of products according to the circumstances (temperature, illumination, etc.) and according to the organisms employed. Just as there are varieties of pigeons and chrysanthemums, race horses and truck animals, dachshounds and greyhounds, so there are varieties of wheats, barleys, grapes and yeasts, differing in yields and differing accordingly in value. But the product of the finest chrysanthemum, grape, or yeast, will be valuable or worthless according to the purity of the processes employed in production. If a good yeast, uncontaminated by molds or bacteria, is added to a clean mash, and the remainder of the process correspondingly handled, a good beer or wine should result; but if a mixed fermentation instead of a yeast fermentation be carried on, the yield will inevitably contain the products—good, indifferent, or positively dangerous—of the other fermenting organisms. These are the reasons for the uncertainties in home baking and home brewing as compared with the dependable, if monotonous and sometimes inadequate, products of commercial establishments.

---

[1] *The Physiology of Plants*, pp. 204-212.

[2] These are alcoholic, producing alcohol, CO_2, and many other substances in smaller amounts.

Whether or not certain plant acids, for example oxalic, malic, citric, are products of oxidative metabolism from sugars, or are by-products in the syntheses leading through the amino acids to the proteins and protoplasm (see p. 27 and also *The Physiology of Plants*, pp. 96, 189, etc.) does not immediately concern us; but, on the other hand, other acids are, without question, the products of oxidative metabolism in animals and plants. For example lactic acid is a product of sugar oxidation whether in human muscle or in cow's milk. Its production and prevention in the latter may thus be demonstrated. Put 2 or 3 cc of skimmed or separated milk into each of six clean test tubes previously cotton plugged and sterilized. Boil the milk in two of these test tubes, cool quickly, and set aside. Into one of these boiled and cooled tubes put a drop of untreated skimmed milk, i.e. infect it. Pasteurize *three* other test tubes of the milk, that is, heat them to a temperaure of 70° C., keep them at this temperature for fifteen minutes, then cool quickly and set with the boiled milk tubes. After the lapse of twenty-four hours pasteurize *two* of these tubes again, cool, and return to their places. Twenty-four hours later pasteurize *one* of these *two* tubes a *third* time, cool, and return. The fifth tube is untouched, and of course care should be taken all along not to withdraw the cotton plugs except as required by the first filling. Note the dates at which the milk sours in these six tubes. It is evident that milk may be sterilized and kept indefinitely, in spite of its high nutrient value and extreme sensitiveness to spoilage, and that a good milk may readily be infected with organisms which will spoil or even more seriously infect it. The diseases of milk and milk products involve fermentations which may be studied in this connection. The degree of acidity, the amount of lactic acid formed, may be determined in the usual ways, or may be merely indicated by the use of litmus paper as a test.

The spoiling of butter is due to the production of butyric acid by the activities of certain other bacteria acting upon the same sugar as in the previous experiment, namely lactose or milk sugar (β-glucose β-galactose). This can be shown by exposing the butter at moderate temperatures to infection from the air, and allowing it to become "rancid."

We can similarly demonstrate the formation of acetic acid from dilute alcohol. Flasks containing 100 cc each of a light beer, light wine, and of sweet cider respectively are cotton plugged and incubated at 35° C. for twenty-four hours. There should by this time be a distinct odor of vinegar, which is crude acetic acid, about the flasks. Analysis will prove that acetic acid is the substance formed. This is accomplished by the partial oxidation in air of the dilute alcohol of these liquors.[1]

There are, however, many metabolic processes actually oxidative in the sense that the proportion of oxygen in the products exceeds that in the antecedent substances, but which take place in the absence of free oxygen. Oxygen is not absorbed, but the proportion of oxygen is higher in the end products than in the carbohydrate and (or) other materials entering the reaction, and energy is released. The processes are called anaerobic or intramolecular respiration and anaerobic fermentation, according to the plants concerned. We can illustrate both very readily. If five soaked peas are peeled and placed in a mercury filled test tube inverted over mercury, the mercury in the tube will presently begin to fall, the space above being filled with gas, which proves to be CO_2, as tests show. Where did this CO_2 come from? Obviously the germinating peas, continuing to respire although in an atmosphere of mercury, have produced the CO_2. (That energy has been released also can be demonstrated by using suitable insulators for the experiment and testing by thermometer.) Similarly fill two long glass tubes (12-15″ long) with a 5% sugar solution colored with Indigo-Carmine, and in which a quarter of a moist yeast cake has been rubbed up. Cover the surface of the sugar solution in one of the tubes with a layer of oil or paraffine one-half inch thick, leaving the other open. After a time the solution will lose its color, in the tube sealed from the air as well as in the other. Indigo-Carmine is colorless when reduced. Hence one infers that oxygen has been taken from it to meet the requirements of the yeast cells; but while a certain, and necessarily small, amount of oxygen has been "donated" by the very small amount of pigment, the usual amounts of CO_2 and alcohol have been produced by the yeast (see experiment p. 117) in spite of the absence of air.

---

[1] *The Physiology of Plants*, pp. 209-10.

In the case of the yeast and in that of the peas just described, in both of which CO_2 has been produced without free oxygen, what has actually happened is the *dehydrogenation* of the carbohydrate.[1] In anaerobic or intramolecular respiration, as in peas under special conditions and probably in all compact or deeper tissues in plants, and in anaerobic fermentations by yeast and many of the bacteria, intramolecular changes take place so that carbon dioxide and alcohol are formed. These intramolecular changes are effected by enzymes formed by the organisms responsible for the respiration and fermentations concerned. To determine these is the province of biochemistry rather than our duty here; [2] but we may well demonstrate the release of energy in alcoholic fermentation. Into clean, sterilized, cotton plugged Dewar flasks pour equal volumes of 10% sugar solution made with clear tap water in which yeast, in the proportion of one moist cake to one liter of water, has been thoroughly mixed. Into one of the flasks drop a few crystals of corrosive sublimate (mercuric bichloride), to kill the yeast, and set all the flasks together, preferably where temperature fluctuations will be as slight as possible. In the course of twenty-four hours the temperature will have risen in the flasks containing live yeast, whereas the temperature in the flask containing only dead yeast will fluctuate with that of the room. The quantity of heat liberated in this fermentation, as indicated in calories, may be calculated in the manner shown in the experiments on heat liberation in respiration (p. 114).

---

[1] *The Physiology of Plants,* p. 190.

[2] Gortner, *op. cit.*

CHAPTER VIII

GROWTH

The plant, having absorbed its food materials, transferred them to the place of manufacture, and elaborated them into foods, may store these foods in buds, tubers, seeds, etc., for future use, or it may use them at once. It may recover from them by oxidative metabolism (respiration) an amount of energy equal to that employed in their manufacture. Or it may form new walls, protoplasm, etc., in *growth.*

The phenomena of growth are so controlled by external conditions, and the reactions to external influences so often show themselves in growth phenomena, that it is difficult and not very intelligent to attempt to study growth without also studying irritability (see the succeeding chapters).

At the outset it will be wise to refresh our ideas as to the *regions, rates,* and *amounts* of growth. Obviously the two latter, and presumably the first also, vary according to circumstances. Then we may study the effects of the different factors of the environment as indicated by the *kind, direction,* etc., of growth.

I. The Growth Regions

If one is not already familiar with the regions of growth in length of the parts of the higher plants—the leaf, stem, and root—one should study them both in the field and in the laboratory, comparing the behavior of annuals, biennials, and perennials. For completeness one should make similar studies of the ferns and mosses, and of the plants still lower, for in these the regions of growth are not always differentiated.

Roots. To determine the region of most active growth in length in ordinary soil roots let us sprout seeds of various larger sorts in pots of damp sand or moist saw dust. We may use peas, beans of such sorts as table, scarlet runner, or *Vicia faba,* and corn. Care must be taken to use saw dust fairly free of resin and tannins, lest the roots be poisoned. And if the air of the laboratory is dry as well as warm, the pots or other dishes containing the sand or saw dust should be covered with glass or with flower pot saucers to prevent excessive drying at the surface. Furthermore, the seeds should be so set that their primary roots will grow straight downwards, unimpeded by pebbles, clods, or clumps. When the roots have attained a length of 1-1.5 cm., select a sufficient number to justify a general conclusion (how large a number should this be?) and mark them with a *harmless* ink at intervals of 1 mm beginning at the tip. The ink must be applied in such a way that it will stick and yet without injury to the root. This may be accomplished by drying the roots, one at a time, by gently wiping them with the torn edge of a piece of thin filter paper, and then immediately applying the ink with a thread or soft brush. The seedlings may now be replaced in damp sand, but holes should be made in the sand of sufficient size and length to receive the seedlings, with their roots pointing downwards and nowhere touching the sand. After 10 hours, and again after 24 hours, measure each zone; record the data; finally plot curves of growth of the entire root to show the relative growth of the different regions, one curve for the 10 hours readings and another for the 24, using zones as abscissæ and the increments of each as ordinates.

Stems. We thus see that the region of maximum growth in length in ordinary soil roots is such that the minimum mass is pushed ahead of it through the resistant soil: the growth is very nearly apical. The supporting power of the soil is great, its resistance no less so. On the contrary, ordinary erect stems grow in a medium of very low supporting power and very slight resistance. To protect the meristem of the root tip from abrasion the thimble like cap forms a cover, renewed as it is worn away. The stem needs no such protective cap, nor does it need to limit its growth in length to a very short zone, growing only for a brief time, and quickly succeeded by another similar zone of transient activity. This contrast with the root becomes clear when we similarly mark the internodes of stems and branches of growing plants indoors and out. We should observe the behaviors of herbaceous plants, and of shrubs, and trees, marking, measuring at frequent intervals, and plotting the curves. From this we see that growth in length is far more general in the stems than it is in the roots of the same plants; except that, if we observe the growth of corn and other grasses, we shall see that the growth of the internodes is basal and not general. We should also consider the relation of these more or less weak (soft) regions of growth upon the general mechanical strength of stems and of roots,[1] and note how this weakness is offset.

Leaves. The growth of the leaves of most land plants is general, as may be shown by marking the under surface of rapidly growing leaves with dots 2 mm apart. The same plants used in studying the growth of roots and stems should have their leaves studied also. Roots and stems are cylindrical, growing at first principally in length, later in thickness, thus segregating both the direction and the time of growth in length and thickness. Leaves, on the other hand, are generally dorsi-ventral organs, thin and flat, growing rapidly and much more nearly simultaneously and equally in length and breadth, but only slightly in thickness. But leaves have far greater diversity of shape than the essentially cylindrical root and stem. Leaves may be composed of stalk and blade, and even stipules, each of which will grow in its own manner, stalk resembling stem, stipules resembling blades, in shape, size, amounts and directions of growth. There is not only coordination, in the separate parts, of the growth of the component tissues (these should be studied in sections and otherwise), but there is also the coordination of the growth of the separate parts in relation to each other; so that stalk, blade and stipules constitute the normal leaf for a given species. This coordination may be accomplished by regulating materials (hormones) or perhaps by heredity; but not enough is now known of the matter to justify more than mention of it here.[1] It will be found by measurement that elongated leaves and circular ones grow in corresponding manner; but *why* this is the case, who can say? As to the growth in thickness of leaf blades measurement is very difficult; obviously the growth is slight in comparison with the growth in length and breadth.

---

[1] *The Physiology of Plants*, pp. 235-249.

In the case of pine needles, growth in length is much more like that of the stems of grasses in that, from a *basal meristem,* the new cells are cut off which elongate and push up the older part of the needle. This can be shown by markings with "waterproof" ink. How long pine needles, and the needle like leaves of other evergreen conifers, continue to grow, and whether they have more than the one growth period, is not now known. Experiment should easily determine this, though observation over the whole life-time of the needles would be required. How many years do pine needles stay on the tree, and how active are they as they age, are other questions unanswered but answerable experimentally.

As to growth other than in length, as shown above in roots and in stems, we can make further measurements. We may determine the diameters, at certain *marked* points, of the roots and stems used above. Micrometer calipers may be used for this purpose; but care must be exercised to exert upon the root or stem no unnecessary, and always the same, pressure whenever a measurement is made. One week later measure the same parts again. The actual increase in diameter is due to the growth, probably also to the formation, of new cells, forming a zone definitely located within the roots or stems. Sections should be cut and studied to determine the location of this zone. One should determine whether growth in length and thickness occurs simultaneously in roots and in stems, as growth in length and width occurs simultaneously in the blades of leaves. And one should compare the behavior of annuals and perennials, of dicotyledonous and monocotyledonous plants, seedlings and older plants, in respect to the regions and times of growth. Comparison of these plants with corresponding studies of the growth regions in sedges, the leaves of onion, water and land plants, ferns, liverworts, mosses, and the algae, will give one comprehensive information on growth in the plant kingdom. One should bear constantly in mind, however, that the collective process called growth is affected and largely controlled by the temperature and other factors of the environment, as studies described in the following chapters will indicate. We may now turn to a determination of growth rates.

---

[1] *The Physiology of Plants,* pp. 282 ff.

II. The Rates of Growth

Observation continued throughout the season, of the plants marked and used in the preceding experiments will show that growth is not continuous or uniform, and that the growth of roots, stems, and leaves is correlated, coordinated, in time and extent. We can supplement the observations previously made by others in which magnifying and self-recording instruments are employed. The simplest instrument we may employ is a horizontal microscope with an eyepiece micrometer. If one carefully transfer a seedling of pea, bean, or corn, with a straight root 1 cm long, from the damp sand or soil or saw dust in which it is growing, to a thin walled bottle with water in the bottom and partly lined with moist filter paper, one may focus the microscope on the tip of the root, setting the microscope at such height that the root tip will be at a convenient point on the micrometer scale. It will be observed, owing to the compound microscope reversing the image, that the root grows up the scale. The growth should be timed, and, knowing the value of the units on the eyepiece micrometer scale, we can calculate the amount and the rate of growth for the time of observation. Seedlings grown with their roots in nutrient solutions (see pp. 88-94), and transferred to the same nutrient solution at the same temperature in bottles or parallel walled small jars (cuvettes), will be more satisfactory because the roots will be less disturbed than when removed from the damp soil or sand or saw dust to damp air. Since the tip of the stem, unlike that of the root, is generally enclosed within one or more leaves, it is not possible to measure the rate of growth in length of the stem as directly as that of the root. But one may, nevertheless, measure the growth of a seedling of corn and of pea, getting, however, the growth figures for both leaves and stem. Do this with corn, marking a spot with India ink on the tip of the first leaf and watching it with the horizontal microscope.

In the above observations care must be taken to get the *full value* of the growth, losing nothing by oblique growth due to the direction of illumination or to other influences.

These observations, especially on stems, can be supplemented and extended over considerable or even long periods by the use of self recording measuring instruments. Many instruments have been devised, the best and most expensive being the so-called dendrographs[1] which can be set on trees and used continuously over a period of years. Others are simpler, less expensive, and less significant, obtainable in the laboratory supply houses.[2]

---

[1] MacDougal, D. T. *Growth in Trees.* Carnegie Institution of Washington. Publication 307, 1921.

[2] *e. g.* Ganong Apparatus, to be had of the Cambridge Botanical Supply Co., Cambridge, Mass.

III. The Kinds of Growth: Tissue Tensions

Growth in thickness and in length of the stem and other organs of larger plants is made up of the component and often very different tissues of these organs. It often happens that these different tissues do not grow at the same rates or at the same times. There may arise, therefore, tensions among the tissues which may even show themselves in movements of the parts. Before we study these, we should know something about the tissues themselves. Therefore careful studies should be made of thin cross sections of (1) branches, and (2) roots, beginning with the season's growth and going back five years, working out—(*a*) The primary bundles. (*b*) The primary and secondary thickenings. (*c*) The character of xylem and of phloem. (*d*) The duration of the growing season. (Does it last as long as the weather permits?) (*e*) The relation of the bundle growth to other growth in the branch or root. (*f*) The relation of vascular and mechanical tissues. (*g*) The annual growth rings.

Having thus seen the tissues of which the root, stem, or branch of a tree is formed, we may proceed to examine the mechanical conditions of the various parts. Unless the different tissues grow at the same rate, those growing more slowly will tend to be stretched, those growing more rapidly to be drawn back; and unless the growth in diameter is also uniform throughout, similar tensions will arise there also. These may be demonstrated in the following ways.

Longitudinal Tensions. One may cut a fresh leaf stalk, say of rhubarb, exactly 20 cm long, and remove a strip of cortex from one side, noting its length exactly and *quickly* (why?) on a sheet of paper. One should immediately complete the removal of the cortex and similarly mark the length of the pith on the sheet of paper. Measuring the marks of cortex and pith will show that neither is 20 cm long, that the pith is more, the cortex less. In other words, the cortex is under tension, the pith under compression in the rhubarb leaf stalk under ordinary conditions. This will be made evident if one split the end of the stalk by a few deep radial cuts. The slices will flare. If one similarly split other leaf stalks, placing one in tap water and another in a 3-5% salt solution, one will find the flaring of the slices respectively more or less than that of the first piece slit. Thus we see that the tensions in the tissues, probably due to unequal growth of the parts, may be aggravated or diminished by changing the water content, the turgidity, of the cells (see pp. 12-15). In fact while the tissues and tissue tensions of leaves, flowers, etc. are strong enough, they will hold themselves normally; but when they lose water, they wilt. We thus see that the mechanical strength of many parts, due to the tissues and tissue tensions, is unequal to the support of the parts unless their water content and turgor are normal.

Transverse Tensions. We may supplement these experiments with others on the scapes of dandelion blooms and on woody stems. Thus one may make two circular cuts, 5 or 6 cm apart, through the bark of willow or any other rapidly growing twig. One will notice the immediate contraction of this portion of bark. If one make a cut lengthwise of this cylinder, one will notice that the edges of the cut separate. Thus we see both longitudinal and transverse tensions in the same tissue. The tensions may be intensified by drying in the air or by plasmolysis, or they may be diminished by wetting, if one choose.

A certain practical significance for human beings attaches to these simple experiments: for we know that the healing of scratches, minor cuts, and even more serious wounds, is facilitated if they are not allowed to dry, as they are prone to do on the desert and in the overheated under-moistened air of our buildings. Our skins are normally under tension; the edges of a cut tend to be pulled apart; but if the cut is kept moist, this tendency is minimized, and healing proceeds from below the more rapidly.

Circumnutation. It is obvious that, unless these tissue-tensions are balanced on the opposite sides of an organ, they may, if sufficient, lead to movements. These movements, because of their regularity were named *circumnutation* by Charles Darwin. They are sometimes said to be spontaneous, but it can be shown that these regular movements, due directly to differences in growth rates in the different tissues or parts of a plant, are indirectly due to those regular influences, such as day and night, light and darkness, which affect growth itself. We may very easily study these circumnutating movements by using the leaves and stems of seedlings, such as sunflower, scarlet runner bean, pea, etc. Darwin also observed the circumnutation of the tips of roots which he kept in moist chambers. One may attach a filament of glass tubing drawn to capillary fineness to the tip of the stem or to a leaf by means of a little wax softened but not melted by heat. If the glass filament is tipped with a bit of sealing wax it will be more visible. At a convenient height above the end of the glass filament fix a clean glass plate horizontally and also another vertically at about the height of the filament. At convenient distances above and in front of the glass plates set other sealing wax tipped capillaries to furnish fixed points for observation. At fifteen minute intervals, sighting through the fixed point and the tip of the filament attached to the plant, make an ink dot upon the glass plate at the spots which are successively in line with the other two points. From the series of dots, and from the distances traversed between times, one can form an idea of the paths, the planes, and the pace of these movements. The record, made on glass, can easily be traced and preserved on paper. The distances actually traversed by the tip of the stem or of the leaf are magnified by the length of the capillary and by the positions of the glass plates; but one may determine this magnification and calculate from the magnified record the length of the path traversed in the unit of time.

From all the previous experiments, and from all of our observations, whether inside the laboratory or outside, it is apparent that external agents produce effects upon objects, animate and inanimate, and upon processes, so-called living processes or functions as well as lifeless ones. In fact external conditions or forces influence growth and every other function of living organisms to such a degree that we cannot understand living beings without also understanding their circumstances and the effects of the one upon the other. This leads us to the subject of the next chapter.

CHAPTER IX

IRRITABILITY

Unless a thing is *sensitive* it cannot in any way be affected by its surroundings. All things are, however, sensitive in greater or less degree. Some things are more, others less, perfectly adjusted to prevailing conditions. We think of living things as adjusting themselves to the conditions under which they live. They do this only to a limited extent, but to do it at all they must be *sensitive* (irritable) and they must *possess the power of reaction.* Living things must adjust themselves as they go along, if at all, to the conditions under which they live; for these conditions change, they cannot be completely anticipated. No amount of sensitiveness would, however, be of any value without the power of reaction. We ordinarily judge the sensitiveness of anything, lifeless as well as living, by the perceptible results or changes which follow a given influence. This is a mistake—a logical as well as physiological mistake—for the perceptible sequelæ of any given influence may indicate only the power of reaction. This may be slight or great. It should never be taken by itself as the evidence of sensitiveness, of irritability. How can one gauge irritability?

The influences to which living things are subject are either *physical* or *chemical;* they are either *formative,* or *directive;* they act constantly and uniformly; or only at times and in unequal degrees. The same influence, like light for example, may act both formatively and directively, but we can distinguish between the two effects. Generally speaking, experiments designed to show formative influences require considerable time and are, therefore, not always convenient. The following experiments must be made with the realization also that the normal organism is subject, though perhaps at different times, to all sorts of influences, not to that one alone which is being studied.[1]

I. The Physical Influences

Among the physical influences to which living things are subject we very naturally think first of the purely *mechanical* ones, such as the wind, waves, currents; but mechanical support, and strain of other kinds than those just now mentioned, may also be considered. For example, what is the mechanical effect on stem, branch, and flower stalk of the setting of the seeds and the growth of a large fruit such as an apple, pear, plum, buckeye, etc.? We may study this out of doors, or in the greenhouse and laboratory, by using plants with quickly ripening fruits. The time of year and the place will determine the selection of plants for experiment; but such heavy flowered plants as Fuchsia, the roses, chrysanthemums, carnations, sweet peas, etc. can be found almost anywhere and at convenient seasons. Selecting a large vigorous plant in full flower, cut off several flowers already opened, others half developed buds, make cross sections of the stalks of the flower clusters and compare the amounts of mechanical tissue at these different stages. Select flowers of as nearly like development, size, etc., as possible; leaving them still growing on the plant, attach some of these to upright sticks and leave others unsupported. Make the difference between support and freedom from support as complete as possible. Some time later cut off both lots and compare them both externally and in cross sections. It will be found that with increasing weight, increasing strain, there will be corresponding increase, development, of the mechanical tissues. Where there is not sufficient to support the flower erect, as in Marechall Niel roses, the flowers and fruits are pendent; but even in this case, with increasing weight, there is increasing tensile strength, as examination of sections will show.[1]

---

[1] *The Physiology of Plants,* pp. 223-28.

We may consider, at this point, the phenomena of "annual rings" as displayed in the trees and shrubs of temperate regions. That they are related to the seasonal changes in weather, etc., and to the corresponding activities of the plants, may be proved by experiment, as well as observed in Nature. In cross and longitudinal sections of stems, branches, and roots one distinguishes the tissues concerned primarily in moving water (the ducts and tracheids in the wood or xylem), the so-called vascular tissues; and those tissues contributing mainly mechanical strength, the fibers and other thick walled elements in xylem and phloem. These are formed seasonally, generally annually. That vascular and mechanical tissues are formed according to circumstances, the preceding experiment suggests; but we can confirm and extend this conclusion by observing not only the effect of increasing load, but also by controlling water movement. We can even use quickly growing annual plants to demonstrate that the water-conducting tissues will vary and will change. Well watered plants not only grow larger than those in water deficiency, both in Nature and in cultivation, but their water conducting tissues correspond. This can be *observed* when one compares cross sections of stems of plants of the same species from different situations; and it can be experimentally produced in experimentally grown annuals and perennials. Furthermore, if one is willing to distinguish between the effects of injury (see pp. 142-4 and *The Physiology of Plants*, pp. 279-95) and the effect of diminished water-consumption and loss, one may early in the season, amputate one half the leaves of a growing branch and at the end of the season compare the wood and bast in a cross section of a branch with undisturbed foliage. By amputating one half the leaves, in the above experiment, one may understand either one half the *number* of leaves or one half of *each* leaf. One may, in fact, try both methods and compare the results as shown in cross sections. In all of these sections—of annuals and perennials—one will find the vascular tissues responding, and recording the environment, an abundance of water either in supply or in consumption being recorded by ample wood, a smaller amount by a smaller ring. When one amputates the leaves, one must weigh them at once and immediately put equal weights upon the places from which they came. These weights, or counter-weights, should if possible be so placed as to exert their full effect, that is, they should exert not only weight but leverage also.

---

[1] *The Physiology of Plants*, p. 242.

We may also experimentally produce two rings in a single season. By complete defoliation at a sufficiently early date in the growing season, by frost or caterpillars or instruments, the growth of xylem and phloem already started will be checked, to be resumed as the second and succeeding crop of leaves begins to come out.[1]

The Influence of Gravity. The foregoing experiments, which show the effect of increasing load, really show the effect of gravity, for if it were not for the pull of gravity, there would not be any increased load or any effect from it. The force of gravity is resisted in two ways; *1st,* by the buoyancy of the medium in which a thing (living or lifeless) is; *2nd,* by the mechanical strength, (the supporting structure) of the thing itself. Thus plants and animals are buoyed up by a force equal to the weight of the volume of fluid which they displace. Aquatics are thus buoyed up by a force about 750 times as great as that which buoys up the parts of land animals and land plants and all other things which are in the air. The skeletal parts, the supporting tissues of the aerial parts of land organisms, must therefore, be more strongly developed than the corresponding parts of aquatics. A comparative study of their anatomy shows this to be the case.[1]

---

[1] Küster, E., *Pathologische Anatomie der Pflanzen* 2d ed. Jena, 1916.

Gravity, then, along with these other mechanical influences affects the amount and kind of growth. In this respect it may be spoken of as a "formative" influence, operating with perfect constancy and uniformity.

Gravity as a "directive" influence seems more familiar, for roots ordinarily grow downward and stems upward, and we attribute their direction of growth to the action of gravity. The phenomenon is as familiar as our own habits of standing, sitting, walking, and balancing. We know that ordinarily we keep our heads up, feet down, and our arms somewhere between. We know by observation, if not by experience, that children learn to stand, walk, and balance themselves, in relation obviously to gravity. That root and shoot respond as definitely as feet and head to the directive influence of gravity may be easily proved. In the first place, although we know that seedlings come up with great regularity in a seed bed of healthy plants, no matter what the position of the seeds which were sowed, we should plant such seeds as those of *Vicia faba,* peas, beans, corn, wheat, oats, etc. in carefully sifted, smooth, and uniformly moist soil, or in saw dust or sphagnum moss. Some of each kind should be so that the tip of the embryo is horizontal, others vertical, others pointing obliquely downward to right and to left, and others obliquely upward to right and left. It should be noted whether the seeds germinate and the seedlings grow to the same height, in the same times, and regardless of position. One may facilitate the observation of the roots by using what is sometimes called a "root cage," namely a box of convenient length and depth but with the sides of glass and so inclined that the roots will become visible by growing against the glass.

If it is felt that even soft and yielding soil or saw dust may hamper root and stem in turning and growing, one may try growing the seedlings in a moist chamber. The trouble with most moist chambers, especially in the dry air of artificially heated buildings, is that they are not sufficiently moist to prevent partial wilting and consequent abnormal behavior of roots and even of stems. Furthermore, pains must be taken to fasten the seeds or seedlings in place in such manner or by such means that they will stay fixed but will not be wounded or otherwise hurt by the procedure. Thus it is necessary to avoid pinning, and one should employ the precautions previously described (see pp. 111 and 123) to ensure the absence of infections, poisonings, etc.

---

[1] Peirce, G. J., "A comparison of land and water plants," *Pop. Sci. Monthly,* July, 1903.

Modifications of the above two experiments will lead us to information as to the sensitiveness of root and stem to the action of gravity, and as to their reaction to it. We may be curious as to many of the details regarding the phenomena of geotropism. We may ask, as implied in the preceding experiments, whether root and stem are most stimulated, or most quickly stimulated, in the horizontal or in the vertical position. We can ascertain this by placing seedlings in a root cage, with root and stem horizontal against the glass, oblique, and vertical but "upside down." Noting the time when the experiment was set up and the moment when the first bending becomes visible, we ascertain the time required for the plant to react to stimulation which has continued for the same length of time. We can distinguish between the time required to induce bending, and the time required to produce bending, in this way. If we expose five plants by keeping them in one position, say in the horizontal, for one, two, three, four, and five minutes respectively, immediately thereafter revolving them on a horizontal axis on a clinostat,[1] we shall see that the growing roots or stems of one or more but not all of these plants will bend even though there is now no up or down side to either root or shoot. Noting the length of time elapsing between exposure and the beginning and the end of the bending, we can recognize the so-called presentation time (i.e. the length of time required to give a stimulus sufficient to produce a visible response, bending) the latent period (i.e. the time during which there is neither stimulus nor visible response), the reaction time (i.e. the time during which bending occurs). There are certain relations at once evident among these times. For example, the longer the presentation time the longer the reaction time and the more pronounced the bending.

---

[1] A clinostat is an instrument for equalizing the exposure of organisms to particular stimuli by revolving them by clock work or otherwise. Such instruments may be obtained through the laboratory supply houses.

By placing the plants for equal times in the diverse positions above named, and putting them immediately thereafter on a clinostat, one can determine the lengths of latent period and reaction time and the extent of the bendings following. Other things being equal, we shall see that the root pointing vertically upward and the stem pointing vertically downward will react most promptly and pronouncedly.

While it is evident from observation and experiment that young roots and stems grow vertically if possible, and that they grow in opposite directions, the means of effecting the positive and negative geotropic growth, and the means of effecting the stimulation of the parts, are not wholly clear. In these experiments if we mark the growing root and shoot with harmless ink, as in the preceding study of growth (pp. 123-4), we shall see that the bending takes place in the region of growth, the greatest bending in the region of greatest growth. In the root, Darwin showed fifty years ago by experiment [1] that the stimulus is received by the root tip and that the reaction is carried out by the growing region immediately behind. Sensitive and responding regions are separate in roots as they are in the ears and feet of men. The stimulus received by certain cells is transmitted to others. How is the stimulus received, and how transmitted? Microscopic examination discloses the presence of starch grains in the cells of the root cap and root tip. These are regarded as *statoliths*, occupying always the lowest position in a cell which they can occupy. If one examine a root tip pointing vertically downward and one lying horizontal, one will find the starch grains along the lowermost wall. Presumably they change their position correspondingly in uninjured cells if the position of the tip is changed. They will thus change the pressures which they exert in the living protoplasm.[1] Other cell contents of other specific gravities will behave similarly.

---

[1] Darwin, Charles and Darwin, Francis. *The Power of Movement in Plants.* London, 1880.

How the stimulus may be transmitted from cell to cell is still a matter of investigation, and the procedures necessary for enlightenment involve skill and equipment, beyond the general conceptions of this book.[2] Suffice it to refer again in this connection to the general idea of a hormone as the specific agent (see p. 125). We must also consider, as part of the general problem of the formative and directive influence of gravity, the coordination of the growth, bendings, and other behavior of the parts and the whole of every living organism. Evidence of this coordination, and its details, can be seen on every hand in garden, field, and wood; and this evidence should be combined with laboratory experience and reading to give one a fair picture of the relations of living organisms to gravity.

Contact. The word contact in plant physiology implies the same as the word touch in animal physiology. We may study exhibitions of the effects of contact in plants and animals, and wonder as to the actual nature of the stimulus, whether it consists in pressure, adhesion, friction, electrical phenomena, or what; but below the surface of examples of stimulus by contact and response or reaction by movement we cannot penetrate at present.[3] But that plants are surprisingly sensi-

[1] *The Physiology of Plants*, pp. 256 ff.

[2] *Ibid.*, pp. 252-55.

[3] *Ibid.*, pp. 261-79.

tive to contact we can readily demonstrate by using tendrils of certain plants and the so-called "sensitive plant" itself.

The classical examples of tendril sensitiveness are furnished by the passion vine (*Passiflora sp.*), *Bryonia, Echinocystis sp.,* etc., but very many other plants—grape, peas, sweet peas, etc. —form tendrils which are only less sensitive or at least react less promptly, than those of the members of the first group. Thus on a warm sunny day, preferably above 25° C. and, if out of doors, nearly windless, mark the position of the tip of a young active tendril, for example a gourd tendril or one of "wild cucumber" which has just straightened. Observe it 15 and again 30 minutes later to determine if it is nutating. Then with a *smooth* pencil or *clean* glass rod gently stroke an active tendril once on the concave side, near the tip. We should mark in how many seconds bending is noticeable. The curvature should be sketched at intervals of two to five minutes, and followed to its maximum and its reversal, if that occurs. One may observe that, not only do tendrils react much or little according to their sensitiveness and power of reaction, but also they react in proportion to the amount of stimulation. For example, suppose we stroke three or more similar tendrils once, twice, thrice respectively—as nearly equally as possible, and in the same regions—and observe the times, extent, and duration of bending. Suppose too that we press or rub one tendril very briefly but very decidedly, and another very gently but for some time. We shall be able to see that the quantity of the stimulus regulates the reaction. Using a stream of water from a wash bottle, or a glass rod coated with gelatine which has cooled enough to set but has not dried, we shall see that the stroked tendrils do not respond by bending. There is no visible response, and we conclude that liquids and moist gelatinous surfaces do not irritate. But if we strew fine granules of sand or other solid upon the moist gelatine, we shall find that touched tendrils promptly turn.

By appropriately modifying these experiments we can ascertain whether the tendrils are equally sensitive on warm or cool, dry or damp days; whether they are equally sensitive at base and tip, on concave and convex sides, and whether light or darkness makes any difference.

If one lightly mark young tendrils with waterproof ink, as previously recommended for the studies of the growth of roots and stems (p. 123), one can see where the growth in length takes place at different times, and under the different conditions of quiet and irritation.

One may make similar studies also of sensitive stems, for instance of dodder, and of leaf stalks, for example of *Solanum jasminoides* (potato vine), and of twining stems. These experiments should also be supplemented by observation and experiment carried out upon plants and plant parts which execute movements, but in which the movements are accomplished, not by reactions to stimuli, but by releases of mechanical tensions. The stamens of species of *Berberis*, for instance, snap forward when touched in a certain manner. On the other hand stigmas of certain plants, *Diplacus* among them, close on being brought into contact with suitable solid objects. This last is a reaction to contact stimulation; the former is merely the release of mechanical tension. This latter is seen also in certain explosive fruits (*Ecballium, Razoumowskia, Arceuthobium,* etc.).

While the release of mechanical tension may be the means of accomplishing certain ends such as the shooting of an arrow, the hurling of a spear, the discharge of certain spores, or seeds, this release may follow a mechanical push or pull, as in the case of the barberry stamen, or it may follow a contact stimulation. This may be made clear by experiment upon the "sensitive plant," *Mimosa pudica,* seedlings or older plants of which may be readily grown in pots if they are not available out of doors. A light touch upon the blade of a leaflet will be followed by more or less general closing of one leaf or even of adjacent leaves, if temperature, etc. be favorable. In fact, both with this plant and with the tendril bearing plants previously studied, one should determine not only the relations of temperature to their sensitiveness and powers of reaction, but one may well try, as has so often been done before, the effects of ether, chloroform and other anæsthetics upon their behavior. In this way we confirm the conclusion that tendrils, "sensitive plants," etc. owe their behavior to the actual sensitiveness of the living things to external influences. Some of these influences act as stimuli, others as sedatives or anæsthetics.

Injury. The effects of injuries, in both animals and plants, are often so long delayed that the connection between cause and effect is difficult to trace. Such lapse of time makes possible the entrance of other factors, so that a given appearance cannot be fully understood, still less attributed to a single cause. In horticulture man resorts to many practices, the first effects of which are obviously harmful, the final advantages unquestionable, but the actual causes very difficult to prove. We may prune fruit trees of various sorts in the usual ways best suited to the given species, but the results we cannot see till the succeeding season; and meantime we realize that the complex of influences in and around the tree has changed, and has correspondingly influenced the consequences of the wounds and amputations which we call pruning.[1] We may, however, make experiments on shorter lived individuals, and so ascertain various results of injury, mechanical, chemical, biological. In this enumeration, however, it should be clearly understood that the injuries of biological origin are due to the mechanical or chemical results of infection or infestation or foraging.

Let us make experiments on common seedlings and potted, garden, or field annuals of suitable form and size to illustrate these assertions. If, for example, we have seedlings of corn, bean, pea or other common annuals and of buckeye (*Aesculus*), pines, etc., planted in "root cages" (p. 136) and boxes or pots, we can decapitate roots and stems of various individuals. If the decapitation of the root take place *very* close to the tip, the tip will be replaced, restored, or "rejuvenated," provided other conditions of temperature, moisture, and aeration be favorable; but if the decapitation be at a point two or three or more millimeters from the tip, no restoration will take place. On the contrary lateral roots will shortly appear at various distances back of the amputation. Such decapitation of stems and branches as is accomplished in "pinching off," "cutting back," and "trimming" results similarly in stimulating the development of lateral buds that otherwise would remain dormant. Thus the stockiness, compactness, of cultivated plants may be promoted, hedges may be produced or walls or fences concealed. When similar results occur in Nature there are generally still further sequelæ; among the lateral branches of stem or root thus stimulated to grow, competition develops, ending in the case of trees with excurrent stems or tap-roots, in the succession of one of the laterals to the leadership of the system. Many cases of this sort are noticeable in field and wood. Thus we find substitutions as well as replacements following mechanical injury.

---

[1] *The Physiology of Plants,* pp. 293-94.

We may follow this still further, studying what happens to the wounds themselves. If one make a clean cut in leaf or stem, the wound will heal, with or without scar, slowly or quickly, according to the method of treatment or neglect following the injury. A burn will heal less quickly than a clean cut. An infected wound may never heal, and chemical injury may be followed by profound changes and only very slow healing.[1] When healing follows injury this is accomplished by revival, in the uninjured cells adjacent to wounds, of activities perhaps long since suspended, such as cell division, growth, and differentiation. These can be seen in microscopic studies of sections of the tissues adjacent to the wounds. Along the cut the injured cells empty, the walls collapse, and thus a partial covering is provided for the living and uninjured cells next behind. Then cells still further from the cut resume their once possessed powers of division and differentiation, a cork cambium forms, finally layers of cork cut off the uninjured from the wounded or killed cells.[1] But though such wounds "heal," in the sense that the plant is once more enclosed in water holding tissues—cork and epidermis—a scar remains.

---

[1] *The Physiology of Plants,* pp. 279 ff.

The substitutions following injury which are of most importance to man are those which the fruit grower seeks to attain by pruning his orchard trees. He aims to increase the proportion of flowering and fruiting branches by cutting off many foliage branches. The same aim actuates the pruning of rose bushes. In other cases the "pinching off" or other amputation is designed to effect a fuller development of what is allowed to persist than would otherwise take place. One need only refer to the florists' practice in the production of giant dahlias and chrysanthemums, for in these cases the lateral buds are removed and the terminal flowers grow correspondingly larger.

II. The Radiant Influences

Besides the physical influences which may be classed as mechanical, and of which the foregoing experiments and discussion are a demonstration, other influences purely physical should also be contemplated. Of these light, heat, and electricity are the most important. The daily change from darkness to light and to darkness again, the yearly change from winter to summer and to winter again, and the apparently utterly irregular but considerable changes in electrical relations, all contribute to the idea that they are of profound importance to living things. It is also obvious on a moment's reflection[2] that they themselves are complex influences, and their effects correspondingly complicated. Thus we have known, from the time when we first saw a rainbow, that what we call light is no single entity, but many entities; and as we have continued to learn, we have found out that light is made up of many components, rays, of different wave lengths, which themselves produce different effects, warming, lighting, bringing about chemical change. We know that heat also may have many forms, as for example the radiant heat which may stream from hot metal or an incandescent filament, or the heat brought to us by the stream of air from the furnace or from the desert or the south. As for electricity, with its rays varying in wave length from X to radio, what may they not do to and within living cells? By experiments we can illuminate the subject, although the shortness of time available may preclude extensive experiment.

---

[1] Wylie, R. B. "Cicatrization of Foliage Leaves," *Bot. Gazette,* 40, 1930.

[2] *The Physiology of Plants,* pp. 296-329.

The Influence of Light. We can recognize that light may exercise formative as well as directive influence on living organisms, provided they are in the formative, growing period when exposed to light. Both influences can be shown very readily by experiment. If one set up a sufficient number of water or other cultures of any convenient plant, such as beans, we may divide these into three sets, keeping one set in darkness, one in the laboratory window, one in the greenhouse or if possible, completely unshaded in the field or garden. The three sets should be at like temperatures. This is perfectly possible at certain seasons anywhere, though not at all times. Keeping, then, seeds, culture material, temperature, humidity, and all other conditions except illumination, the same for the three sets, we shall presently be able to see that shape, size, character, and direction of growth are all influenced by light. The seedlings with slenderest stems, longest internodes, smallest leaves, and least color (if any) are those growing in darkness. Those with stoutest most compact stems, largest and greenest leaves are the ones in the most light. Those on the window sill are midway as to size, color, etc., but they are bent toward the window through which the light comes. Similarly, if we imbed two potatoes erect and side by side in a pot of moist sand, covering one with a clear colorless glass jar and the other with a similar jar made opaque with Japalac or other paint or lacquer, we shall presently see that the potato sprouts are unlike in size, color, development from the start, and that the only difference in the circumstances of the two is in the presence or absence of light. Pumpkin or squash seeds may also be planted in pots of damp soil, saw dust, or moss and, except that one pot is kept in darkness and the other in the light, the treatment is the same. When the seedlings are a few inches long they should be marked with dots of India ink equally spaced. Subsequent changes in relative positions will indicate the directions and amounts of growth in the stems and leaves marked. If the cultures can be continued long enough, marked differences in the time and abundance of flowering will be evident between the plants in full and in half light. Upon this phenomenon depends much of horticultural and floricultural practice—"forcing" for example, and some of the advantages of pruning—and the distribution of plants in agriculture and in Nature.[1] We may, if we choose, penetrate slightly below the surface of plant behavior in response to light by sowing fern spores on damp soil, or filter paper moistened by nutrient solution, or the surface of a nutrient solution, and so placing the cultures that they will receive light from one side only. We shall see, when the spores germinate, that they send out a germ tube from the lighted side of the spore and that the division walls form at right angles to the light.

As to the directive (*tropic*) influence of light every one's observations contribute evidence. We know that stems ordinarily bend toward the light. They are said to be positively phototropic. Most leaves are spread at right angles to the light. They are said to be diaphototropic. Organs which, like roots, grow in darkness, may show reactions to light, but most roots are not phototropic, the direction in which they grow being determined by other factors of the environment, mainly gravity. These statements can be substantiated by experiments.

---

[1] *The Physiology of Plants,* pp. 305 ff.

Seeds or seedlings of white and of black mustard may be set in holes in sheet cork, or on sufficiently fine cheesecloth, on a suitable nutrient solution in a tumbler or beaker. The short roots should dip into the solution. If they are kept in darkness until roots and stems are reacting only to gravity—as a matter of convenience, from a few hours to one day—and then exposed to a beam of light coming from one direction only, it will presently be found that the stems of both species of mustard will turn toward the light by bending, and that the roots of one species will turn away from the light while the other continues to grow straight. We should determine the temperature, the time of exposure, the time when bending first becomes evident, the place where the bending occurs in the stem and in the root, and the parts of stem and root most sensitive to light. For this last purpose other seedlings than those of mustard give more striking results.

Sow oats in pots *filled to the rim* with good soil and allow to grow in darkness until the seedlings are about 2 cm tall. Meantime prepare small opaque caps of "tin foil." By rolling squares of tin foil, cut to about 6 x 6 mm, over a wire about the same diameter as the oat seedlings, one obtains short tubes. One half of these should be bent over and pinched shut at one end, the other half remain open. Over as many seedlings slip the open tubes, and over as many more the closed caps. The pots are then exposed in a dark box to a beam of light from one direction only, as in the above experiment. Exposing one pot for one hour, and other pots for longer and shorter times, and returning them to darkness, one may determine *both,* (1) that the tip of the coleoptile of the seedling is the receptive region and the bending takes place elsewhere, and (2) that, as in the case of gravity, there are definite presentation times, latent periods, reaction times, and relations of quantity and duration of stimulus to extent and duration of bending.

We may also wonder, since light consists of radiant energy of different wave lengths, whether these different wave lengths are equally and perhaps even similarly stimulating. We know, for instance, that the dark rays from an incandescent carbon filament of an electric lamp can be used to warm the human skin and to produce certain corresponding results in redness or relief from pain, etc.; and we also know that the ultra violet rays from a mercury vapor lamp can be used to stimulate the skin and the underlying tissues, resulting in brownness of the skin and improvement in metabolism and growth. To show whether these same rays, at the red and at the violet ends of the spectrum, affect plants we may enclose an incandescent "Mazda" bulb in a suitable box with a small window on each side, one covered with a square of monochromatic red glass and the one on the opposite side with a square of monochromatic blue glass. Setting pots of oat or mustard or other seedlings at the same distance from the red and from the blue glasses (and otherwise in darkness) we shall presently see that the bending occurs only on the blue side. It is, then, the chemical rather than the heat or the luminous rays composing daylight which stimulate the cells in such fashion that their reaction produces bending toward the light. We may, in fact, by growing three pots of mustard seedlings otherwise alike—one in complete darkness, one in red light, one in blue or violet light—ascertain that, in general, plants grow least in red light, longer in blue, longest in none (see pp. 145-6). If we wonder how much of this result is due to unequal amounts of energy passing through red and the blue glasses, we can determine the quantities of energy emanating from the two sides of the black box by means of a thermal couple and the readings of a galvanometer. From this we can see that it is not the quantities of radiant energy but the wave lengths which determine the behavior of plants and of other living things.

To show the lag between phototropic stimulus and response we may use oat or mustard or other convenient seedlings which have been in the dark long enough to recover from any previous stimulation (how long should this be?). Exposing one set of these to one sided illumination in a dark room for a quarter hour, another for a half hour, and others for longer times, we may return them to the dark room and determine how long after stimulation the bending begins, how long it continues, the amount (final angle) of bending, the relations of quantity of stimulus and amount of reaction, etc.

Furthermore, one should ascertain, as in the case of geotropically stimulated roots, by marking the stems suitably, where the bending takes place, whether this is accomplished by shortening or by growth. And we can similarly ascertain, in the case of the light sensitive roots of mustard, that it is the same growing region immediately behind the root tip that executes the bending in response to light as well as to gravity. We may infer and, if we choose, prove that the tip of the root is sensitive to light also.

Into the case of diaphototropic leaves we can gain some insight by experimenting upon young nasturtium (*Tropæolum*) plants in pots. Geraniums and many others will do as well. The blades of some of the leaves should be completely and neatly covered with tin foil. Other leaves should have only the stalks enclosed in foil. Still others should be uncovered. These plants should be exposed to light coming from one direction only while another pot should be kept in darkness. The exposure to light and the stay in the dark may be for twenty-four hours, or less if more convenient. At the end of this time note the positions of all the leaves, and determine the relation between light direction and the position of the blades of leaves exposed to it. We can also ascertain by this form of experiment the length of exposure required to produce a visible effect either in position or change of position, the presentation time, latent period, and all the other features exhibited by the preceding experiments. We may also in experiment on potted or boxed plants ("in flats") repeat what we so strikingly see out of doors, namely the change in position of leaves with the movement of the sun from its rising to its setting position. The relation of this diaphototropic position to photosynthesis should be considered.

Plants are normally subject to variations of illumination accompanying the alternations of day and night. Certain plants exhibit conspicuous responses under these conditions. The changes in positions of the leaves of certain plants, the movements, misleadingly called "sleep movements," are easily observed at night fall in such plants as clover, oxalis, etc. Such changes are due to variations in illumination, and have been called "nyctitropic" movements; but this term is objectionable as the changes are not due to growth changes but to turgor changes. In many cases the nocturnal position of the leaves may be induced by putting the plant in darkness. A much better term for the response is *photeolic*. The sensitive plant, *Mimosa pudica*, is a conspicuous example which exhibits photeolic movements to a marked degree. Seedlings of this plant may readily be grown in pots, and one may also use, out of doors or in the laboratory, oxalis and other plants the leaves or flowers (California poppy, four o'clock, etc., etc.) of which open and close with increase or decrease in light. If clover, oxalis, or bean plants, out of doors or inside, are covered at mid day with a box or flower pot the leaves and leaflets will change, despite the hour, to the nocturnal position. If these plants are kept in darkness for forty-eight hours their leaves will have certain positions which will change whether they are thereupon placed in bright diffused light or in light which comes mainly or entirely from one direction.

The Influence of Heat. Every process in Nature requires, as one of the conditions for its occurrence, a certain minimum temperature. Many processes are accelerated by higher temperatures and delayed by lower ones. These commonplaces of the chemical and physical laboratory are no less true in the physiological. A certain minimum warmth is required for the living, breathing, growing, and reproducing of any living organism, plant and animal; and up to certain temperatures, characteristic of each species, the vital processes accelerate. These temperatures are called optimal. Temperatures rising still higher delay one or more of the vital processes and will finally stop them. These results, so far as cells are concerned, we have seen under the microscope (pp. 10-11). They may be observed in whole organisms with the naked eye and under field or other natural conditions. But it should be noted at once that changes in temperature ordinarily entail other changes. These must be recognized and reckoned with. Among such secondary or induced changes should be mentioned increased evaporation, decreased soil water and hence possible decreased absorption and consequent wilting, speeded water movement in the body of the plant. This is particularly true of the vascular plants. But we shall see also that the changes in water content of cells, of organs, and of organisms affect their relations to heat.

These various categorical statements may be readily confirmed. Thus, suppose we place equal quantities of sodium carbonate in equal volumes of water, each different volume of water having a different temperature. The rates of solution at the different temperatures are also different. If now we add equal quantities of dilute HCl to the different solutions with their different temperatures, we shall have unequal destruction of the sodium carbonate and unequal bubbling off of CO_2. Also we must take into account that CO_2 is more soluble in cold than in warm water. We will now select equal numbers of air-dry pea seeds of the same variety, size, color, etc., dividing them into ten lots. Five peas of each of these lots we put into equal volumes of water in four test tubes, and place these test tubes one in a freezing mixture,[1] another in ice water, another in water at room temperature, and the fourth in an incubator at 36° C. How rapidly do these peas manifestly take up water and swell, and at what times after starting the experiment do they germinate? The other six lots of peas should be set in damp saw dust or sphagnum to sprout, and when the radicles have just begun to appear, they should be distributed into test tubes to be put like the others into the freezing mixture, the ice water, kept at room temperature, and incubated at 36° C. What happens to the sprouting seeds is manifestly different from what follows exposing the air-dry seeds to this range of temperatures, and we can only infer from this that the sensitiveness, irritability, balance, or whatever we choose to call it, of air-dry and dormant seeds is decidedly lower than of the active sprouting seeds. From our work on living cells (pp. 6-31) we recognize the importance of the *system* which consists of proteins, carbohydrates, lipoids, electrolytes, and water, and we realize that insufficient proportions of water, or of any other constituents of the system, affect the relations of the system to heat, as to other factors of the environment.

---

[1] For example:

1 Solid CO_2 + 1 Ether yield—100°C.
1 Solid CO_2 + 1 Alcohol—72°C.
3 Dry crystals $CaCl_2$ + 2 powdered ice yield about—50°C.

The influence of heat, temperature, as thus indicated may be said to be, in accordance with the language thus far used, formative. We may suspect that radiant heat, as it streams from a heated surface, a steam-pipe, or a lamp of any sort, may also stimulate growth and direct it, but since other factors than radiant heat are involved in all the examples that have been observed and recorded, we may content ourselves with realizing the importance of heat as a factor of the environment and its significance in relation to growth.[1] Thermotropism is doubtful among plants, whatever may be said about the movements of animals, human and other, about a stove or camp fire!

The Chemical Influences. Under the term Chemical Influences may be included water and all of those other substances which, by reason of their composition and affinities, affect living organisms. Many of these substances are, like water, constituents of that system or structure, living protoplasm. Others, by combining with water or with other constituents of the protoplasm also affect it more or less profoundly, more or less visibly. So far as the effect of water and of other substances upon individual cells is concerned we may refer to our previous work under the microscope (pp. 6-15). Bearing in mind that the effects which the whole organism may exhibit are the collective results of the reactions of cells to stimuli, we may supplement our previous results and conceptions by observation out of doors and experiments in the laboratory.

---

[1] *The Physiology of Plants*, pp. 329-33.

The formative influence of water is exhibited everywhere. We need only compare the appearance and structure of water and land plants to realize this. And if one suspect that a part of the differences between the two may be due to differences in their position in a classification or in a scale of development, let one compare the submersed, floating, and land species of the same or similar species, genera, and families. Thus one should compare the appearance, structure of the leaves and stems, etc. of buttercup (*Ranunculus*), the dry terrestrial with the meadow, swamp, and aquatic species; the aquatic mare's tail (*Hippuris*) and Milfoil (*Myriophyllum*) with the *Aralias* of wet and shady places in woods and the *Umbelliferæ*, some of which occur even in very dry situations. Plants are, therefore, described as having a hydrophytic, mesophytic, and xerophytic *habit* respectively.

In certain respects perennials change, as to foliage, from season to season, according to rainfall, humidity, etc., and in certain annuals results are obtainable within the time ordinarily available for experiment.[1] Thus if one plant Winsor bean seeds in the same soil and subject them to otherwise the same treatment, keeping one half, however, in dry air and the other half in moist—one half out of doors or in a dry greenhouse and the other in a similar greenhouse kept much more moist—one will find striking differences in the leaves of adult plants; for the out of doors plants will have more compact leaves with stomata mainly or only on the under side, whereas the greenhouse plants will have less compact leaves with equal number of stomata on their upper and lower sides.

---

[1] *The Physiology of Plants,* pp. 335-6.

One can, furthermore, experiment also with the aquatic buttercups, water-lilies, and similar plants, showing that the length of stem, length of leaf-stalk, shape and other characters of the leaves, depend upon the depth of water, and whether the leaves are submersed or floating.

One may experiment also with plants which, for example, bear two kinds of leaves, a cushion or rosette at the base and smaller leaves above spaced at much greater distances (for example mullein, *Verbascum,* tarweed, *Hemizonia,* etc.). If these plants, which produce their basal leaves early in the season, when there is an ample supply of water in soil and air, are grown indefinitely in an abundance of moisture they will react accordingly—the mullein leaves will be far less hairy and larger where water loss from them is moderate; and the tarweeds will not be the scrawny sticky plants which one finds in the summer hay fields.

One may experimentally stunt the growth of plants by limiting the quantity of water. This is done for commercial reasons in certain instances and with great skill, as the Japanese dwarf trees show. One may control the water absorbing, conducting, and losing tissues within the hereditary limits, and in both annuals and perennials, by regulating the water supply. One may stunt or exaggerate the height of plants at will, though only within limits. If one plant sweet peas of the same strain —"a pure line"—in the same soil and give them as nearly identical surroundings as possible except that one half are in moist soil and damp air, and the other in moist soil and dry air, one will find that the latter stop growing first, are shorter, and perhaps bear more flowers.[1] In fact, one may infer that in many instances, water regulates the height of plants. In sweet peas the efficiency of the conducting system would seem to be the means by which water exerts its formative influence.

If one sprout mustard, wheat, or other quickly germinating seeds or grains in boiled and cooled water, in damp compact soil, saw dust, and moss, and in damp cloth or filter paper under a tumbler, one will find that the difference in the quantities of water appear to be directly connected with the number, length, and time of appearance of the root hairs and the growth of the roots. But if one be careful to supply an adequate amount of water in every instance, then the differences in the quantities of water entail differences in the plants which are due not to the water directly but directly to the differences in oxygen supply; for from the moist air the plants may be able to supply their water requirements as completely and easily as from water or damp compact soil, but from the large volume of air they obtain oxygen much more rapidly than from boiled water or any of the other media in our list. That this is correct is indicated by the different numbers, etc. of root hairs in these different substrata, the fewest root hairs being in the oxygen-free boiled water, then in compact soil, in saw dust, in moss, and finally most abundantly in moist air.

This last experiment leads us to see that, in addition to water, many other substances are or may be involved as formative influences. We have already observed this in connection with the cell studies (pp. 9-15) previously made and in nutrition (pp. 89-94). We may now extend some of the observations made on single living cells 20 to whole plants or to branches. We saw that the rate of protoplasmic streaming could be raised or lowered according to the amount of ether added to the water in which the cells were mounted. Similarly we can "break" the so-called resting period of many plants. For example, one may cut off under water in the usual manner, and during the dormant period (October-April?), flowering branches of peach, cherry, lilac, Japanese quince, etc. and subject these to ether vapor under a bell glass or in other tightly closed vessel for twenty-four hours or more. Approximately 0.5 cc of ether per 1000 cc of air in the vessel should be put in an open dish and allowed to evaporate in the tightly closed space. Similar branches should be subjected to the same treatment but without etherizing. It will be found that the latter open their buds much later.

---

[1] *The Physiology of Plants,* pp. 307-13.

Ether is thus shown to stimulate growth for which provision has already been made. That the kind of growth may be influenced by chemical treatment may well be conceived, but apart from the familiar practices in soil manipulation one cannot go far.

One may make significant experiments with pollens and with spores, however. If one prepare solutions of different sorts and sow pollens upon them one may see that the germination and growth of pollen are favored by certain procedures. A few cc of tap water should be poured into heavy watch glasses (Syracuse). To the first of these add nothing, to the second 5% cane sugar, to the third 10%, to the fourth, fifth, and sixth 7½% cane sugar, to the fifth a few granules of cement dust or other mild alkali, and to the sixth enough oxalic acid to make the liquid slightly acid to blue litmus paper. Each dish may be covered with glass to reduce evaporation, and allowed to stand at room temperature. In the course of a few hours one will be able to observe that different pollens require different proportions of water—some require no more moisture for germination than may be afforded them by breathing on a glass slide upon which they have been dusted and then covering with a cover glass—and that the reaction (pH) of the solution is manifestly also important. We may conclude also that those natural processes and those human interventions (smoke, dust, drainage, etc.) which affect the composition of air, soil, and the waters, will and must influence the growth of all things living. For example, factory smoke makes poor fertilizer!

Turning from the formative to the directive influence of water and of other substances we can demonstrate this in many ways. We can show that the direction of growth of roots is influenced by water. Thus we may cover the outside of a funnel with wet filter paper, plug the neck loosely with cotton and fill with sand, heaped up. Imbed grains of corn in the sand with their points projecting over the edge of the funnel, and cover with wet filter paper. Set the stem of the funnel in a wide-mouthed bottle filled with water. Place all under a bell-jar in the dark. The roots as they develop will turn over the edge of the funnel but, unless the air under the bell-jar is too moist,[1] the roots will not grow vertically downward but will turn still further and, remaining in contact with the wet filter paper, will grow obliquely downward. This direction is the resultant of the simultaneous operation of two stimuli, the stimulus of gravity and the stimulus of water. In the soil the natural or artificial irrigation may result in the top layers being moister than the lower. In this case gravity and water work oppositely. The branch roots record this by their direction and perhaps more especially by their location; for in such soils it often happens that the roots are massed near the surface. One may see the same thing near the borders of streams and ditches, in lawns and garden bed, etc.

The directive influence of a variety of substances upon the growth of pollen grains and fungus filaments, and upon the locomotion of sperms, bacteria, etc. may be illustrated in the following ways. To a hot 10% solution of sugar add ½% gelatine. See that the solution is only slightly acid, neutralizing if necessary with Na_2CO_3. Place a drop of this solution on a clean slide, dust with ripe sweet-pea or other pollen, cover neatly (so that there are no air bubbles and that the liquid does not extend beyond the edge of the cover glass) and place in a moist chamber. Germination should take place within two hours. Notice the effect, if any, of distance from the air upon germination and subsequent growth. One should also try the effect of the stigma and of the ovules of the same species upon the direction of growth of the pollen tubes. The bending toward the edge of the cover glass is a reaction to the stimulus of oxygen. The bending is said to be aerotropic but it is merely an exhibition of the more definite and at the same time more general phenomenon of chemotropism. Similarly one may cultivate the spores of parasitic fungi in similar media and demonstrate that the direction of their germ tubes will be influenced by pieces of leaf or of epidermis placed on the culture. In these cases it must be substances diffusing from the tissue which stimulate the growing fungi. This may be demonstrated by making a strong leaf decoction, filling a glass capillary with this and placing this capillary in the culture. The response of the growing fungus hyphae will be the same, whether living leaf or lifeless solution be used.

---

[1] One must realize that if there is an equal distribution of water in air and paper the roots will be subject to only one directive stimulus, namely gravity, and will grow accordingly. If necessary, ventilate the bell-jar by keeping the edge slightly raised.

Chemotaxis may similarly be demonstrated, for by filling glass capillaries with sugar solution, beef tea, dilute alcohol, dilute malic acid, tartaric acid, etc. and introducing these into drops of water on slides under the microscope one can see the effects of these substances as they diffuse from the capillaries into the drops of water in which the sperms of fern, moss, liverwort are swimming, or in which one has suitable species of motile bacteria. Dilute alcohol will repel, most of the other substances attract. One has, therefore, negative or positive chemotaxis.[1]

Thus we come, near the end of our course, to the point of view with which we started, namely that the effects which we may observe with unaided eyes can be understood only as we know and understand the effects of individual stimuli upon individual cells. The behavior of individual cells consequent upon the operation of given stimuli upon them is the cause of the cumulative effects visible to the naked eye. Our physiology therefore becomes the physiology of cells, or the physiology of protoplasm, rather than of heart, nerves, muscles, leaves, stems, and roots. The living organism, being a larger or smaller mass of coordinated cells or coordinated protoplasm makes a response which is the sum or the resultant of the responses of its components to a given stimulus or to a given complex of stimuli or a given environment. Given the mechanism—the protoplasm, the cells, the tissues—the responses will correspond with the stimuli.

---

[1] *The Physiology of Plants,* pp. 346-8.

CHAPTER X

REPRODUCTION

Experiments on the physiology of reproduction in plants involve the expenditure of so much time and the employment of such techniques that they belong rather in a course in Genetics than in one in Physiology. It should be remarked, however, that in the foregoing chapter (pp. 145-50) experiments are described which throw light upon the causes (stimuli) which lead to blooming and fruiting, and which guide the pollen tube and the sperms to the eggs which they are to fertilize. As to the details of such fertilization the cytologist with his technique, and possibly the biochemist with his, must determine; for chromosomes, genes, proteins, enzyms, and hormones require methods of study beyond the scope of this book. From the preceding experiments we have gained ideas as to the effect of environment upon the materials which we have studied. From morphologist and cytologist we gain ideas as to the organizations involved. From the genetist we may learn the rôles played by the different parts in the organizations. We know that living things ordinarily provide their own successors, and that there is continuity of substance and of organization between the generations; and to the fundamentals of living and of succession the inquisitiveness of our successors may be able to penetrate. For this the future will provide the questions and the means of answer.

INDEX

A

Absorption, 34-50, 151
Acetic acid, 27, 120
Acid, 23, 25-29
Acid fuchsin, 35, 60
Aesculus, 2
Agave, 58
Aggregate, 18
Alcohol, 15-17, 117-121
Algae, 48, 91
Alkaline, 23, 24, 25, 26
Alkanna, 21, 87
Aluminum, 74, 91
Amino acids, 88, 96, 106, 118
Ammonium, 24, 25, 75, 89, 90, 108
Amylase, 97, 102, 104
Anaerobic, 120-121
Anaesthetics, 142
Anilin dyes, 35
Annual rings, 128, 134-135
Antagonism, 92
Antiseptic, 31, 111-112
Aphids, 58
Arceuthobium, 141
Ascent of sap, 67
Aseptic, 112
Ash constituents, 21, 91-94
Asparagin, 96
Atoms, 30

B

Bacteria, 32, 88, 101, 106, 108-109
Bakhuyzen, 7, 93
Balanced solution, 92
Barium, 36, 40, 42, 115-118
Barometric, 66
Becking, 7
Beets, 38
Belling, 6
Benedict's solution, 19, 42, 57, 58, 71, 86-87, 96-97, 103-104
Benzene, 20
Berberis, 141
Bicarbonates, 76
Biuret test, 106
Bleeding, 57-59, 63-64
Bog plants, 48
Bordeaux mixture, 91
Bordered pits, 63
Boron, 22, 74
Bose, 67
Bread, 118
Brownian movement, 7, 25
Bryonia, 140

C

Caffeine, 32
Calcium, 21, 24, 25, 27, 75, 91-92
Calipers, 45
Calorie, 114
Camera, 41, 44
Cannon, 80-81
Carbohydrates, 6, 19, 22, 31, 39, 44, 74, 116, 120, 152
Carbolic acid, 32, 111
Carbon, 75
Carbon dioxide, 76-87, 108, 115
Carica, 105
Carotin, 83
Castor bean, 98, 104
Catalyser, 103
Cell, 1-33
Cell sap, 49
Cellulose, 19, 37, 43-44, 104
Century plant, 58
Ceratophyllum, 76, 78
Chemotaxis, 157
Chemotropism, 158
Chinese ink, 60

Chloride of lime, 32
Chloroform, 20, 87, 98, 142
Chlorophyll, 78-85, 95
Chromatophores, 83
Chromic acid, 29
Chromosome, 29, 160
Chrysanthemum, 2, 144
Circumnutation, 130
Citric acid, 27, 118
Cladophora, 12-13
Coagulation, 11, 15-16, 152
Cobalt, 53
Cocaine, 32
Cohesion, 8
Colander, 37
Coleoptile, 147
Colloid, 29-31, 39, 44
Colorimetric, 26
Competition, 143
Conducting tissues, 59-69, 155
Conifers, 60-64
Contact, 49, 139-142
Continuity, 12
Copper, 35, 42-43, 74, 90, 91, 111
Coordination, 125-126, 139, 159
Cork, 6, 59
Corn starch, 61
Correlation, 126, 159
Corrosive sublimate, 111, 121
Counter-irritant, 32
Cranberries, 27
Critical temperature, 11
Cucurbita, 2
Cutin, 5
Cytoplasmic membrane, 38-39

D

Dahlia, 97, 144
Dandelion, 129
Dark field, 6-7, 30
Darwin, 130, 138
Dehydrogenation, 121
Dendrograph, 127
Desert plants, 48
Dew, 57
Dewar, 109
Diaphototropic, 146, 150
Diastase, 97, 101, 104
Diatoms, 87
Diffusion, 34-41, 60
Digestion, 98, 100-107
Diplacus, 141
Directive, 132, 136, 157
Dissociation, 22, 25, 30
Distilled water, 89
Dixon, 65
Dodder, 88
Donnan, 45-46
Dormancy, 113, 156
Duco cement, 68
Ducts, 60-71, 96

E

Ecballium, 141
Echinocystis, 67, 140
Electrical charges, 22, 46
Electrolytes, 6, 22, 23, 31, 74, 152
Elodea, 6, 7, 9, 12, 24, 76, 78
Emulsion, 7, 26
Energy, 108
Enzymes, 97-98, 102-104, 160
Eosin, 35, 39
Epidermis, 47, 50, 53-54, 68
Equilibrium, 49
Equisetum, 6
Ether, 20, 87, 98, 142, 155
Evaporation, 13, 51, 64, 70, 151
Excrete, 42

F

Fats, 20, 86, 104, 116
Fehling's solution, 42, 57, 58, 71, 86-87, 103-104
Fermentation, 117
Ferns, 48, 146
Fertilizer, 90-91, 93
Field corn, 98, 104
Findlay, 30
Fixation, 29
Flaccidity, 14
Flavors, 98
Flowering, 91, 146
Foods, 70-122
Forcing, 146
Formalin, 32, 111
Formative, 132, 136, 152-153, 155, 157
Formic acid, 86

Fragrances, 98
Freezing, 49, 151
Fuchsia, 133
Fucus, 71
Fungi, 88, 109, 111-112, 116-118

G

Gage, 7
Galvanometer, 68
Gasoline, 20
Gel, 7
Gene, 29, 160
Genetics, 160
Geotropism, 135-139
Geranium, 52, 82
Gericke, 93
Glucose, 42, 86, 103
Gram's Iodine, 4, 15
Gravity, 65, 135-139
Growth, 43, 95, 122-131
Guard cells, 53-56
Guttation, 57-59

H

Hairs, 2 ff., 46-50
Healing, 143-144
Heat, 108, 150-152
Hemizonia, 94, 154
Hemp, 115
Heredity, 125
Hexylresorcinol, 32
Hippuris, 153
Holding capacity, 48
Honey dew, 58
Horizontal microscope, 67, 126
Hormones, 125, 139, 160
Humid, 49
Humus, 93
Hydration, 8
Hydrochloric acid, 27, 105
Hydrogen, 26, 32
Hydrostatic pressure, 44, 65
Hydrotropism, 157
Hydroxyl, 26
Hypertonic, 13
Hypotonic, 13

I

Imbibition, 44-45, 64, 75
Impatiens, 67
India ink, 60, 127
Indigo carmine, 120
Injury, 142-144
Interface, 36-37
Intermediates, 116
Intramolecular, 120-121
Inulin, 4, 15-17, 31, 61, 78, 82, 84, 86, 98
Iodoform, 118
Ions, 22, 23, 28, 30, 45-46
Iron, 75, 91-92, 108
Irrigation, 48
Irritability, 122, 132-159
Isotonic, 13, 41

J

Jerusalem artichoke, 97

K

Kelps, 71
Kinetic, 34, 41

L

Lactic acid, 119
Lag, 148
Laminaria, 45
Leaf mold, 92
Lenticels, 52-53
Light, 144-150
Light green, 35, 60
Lignin, 5
Lipases, 98, 104
Lipoids, 6, 20, 21, 22, 31, 74, 152
Loam, 93
Lugol's solution, 15

M

Magnesium, 21, 75, 91-92
Malic acid, 27, 118
Maltose, 97, 103
Manganese, 22, 74, 91
Manure, 90-91, 93

Maple, 58
Marsh plants, 49
Mechanics, 12, 128, 133, 141
Membrane, 21, 37-50, 63
Mercurochrome, 32
Mercury, 66, 120
Meristem, 123-125
Mestre, 85
Metabolism, 28, 33, 95, 108-121
Methylen blue, 39
Mice, 113
Millet, 48
Milk, 119
Millon's reagent, 99, 106
Mimosa, 141-142, 150
Mineral salts, 21-24, 93
Moist chamber, 101, 137
Molecules, 29, 30
Molisch, 21
Montia, 67, 94
Morrow, 20, 49
Morse, 43
Motile, 36
Movement, 9, 17, 51-72, 128, 150, 155
Mustard, 91
Myriophyllum, 76, 153

N

Nasturtium, 52, 55, 57, 149
Natural waters, 92
Negative pressure, 64
Nicotine, 32
Nitrate, 88
Nitric acid, 21
Normal solution, 29
Normal temperature, 109
Nucleus, 29
Nyctitropic, 150

O

Oats, 48, 147
Oils, 20, 86, 104, 116
Oleander, 82
Onion, 97, 126
Osmic acid, 21, 29, 87
Osmometer, 40
Osmosis, 4, 13, 39-50, 96
Overton, 68
Oxidative metabolism, 28
Oxalic acid, 27-28, 118, 156
Oxygen, 46, 80, 108, 120

P

Papoid, 105
Parasites, 49, 88
Partial pressure, 41
Passiflora, 140
Pasteurize, 119
Pectin, 103
Pelargonium, 2
Pentathionic acid, 112
Pepsin, 104-105
Permeability, 21, 38, 42
Pfeffer, 43
Phaseolus, 57
Phloem, 63, 71, 96, 128, 134
Phloroglucin, 5
Phosphorus, 21, 75, 91
Photeolic, 150
Photosynthesis, 78-87, 95-97, 104, 150
Phototropism, 146-150
Picric acid, 29
Pinching off, 143-144
Pineapple, 105
Plasmolysis, 14, 24, 129
Poison, 18
Pollution, 25, 83, 156
Potassium, 13, 15, 21, 24, 32, 35, 36, 42-43, 48, 75, 89, 91-92, 115-118
Potato, 98, 100-101
Potted plants, 77
Press juice, 31
Pressure, 3, 12
Proteins, 6, 7, 18, 22, 24, 25, 38, 74, 98-99, 106, 118, 152, 160
Protoplasm, 6-24, 74, 118, 139, 153, 155, 159
Protoplast, 13, 37
Pruning, 142-146
Puffed rice, 61
Puffed wheat, 61
Pulque, 58

R

Radish, 91
Ranunculus, 2, 153

Raspail's reagent, 20, 99
Rattan, 85
Razoumowskia, 141
Reproduction, 160
Reserve cellulose, 97
Respiration, 28, 103, 108-121
Resting period, 155
Rhubarb, 27, 128
Rigor, 11
Ripening, 83
Root cage, 136-138, 142
Root hairs, 46-50, 59
Roots, 123

S

Sachs, 12
Salamander, 113
Saliva, 101
Salts, 6, 7, 22, 28, 35, 39
Salt Marsh plants, 42, 48
Sap, 13
Saprophytes, 49, 88
Scott, 94
Sea weeds, 42
Semipermeable, 38
Sieve tubes, 71, 96
Silicon, 6, 74, 91
Silver nitrate, 32
Sleep movements, 150
Sodium, 6, 7, 22, 24, 151
Soil, 47-52, 92
Sol, 7
Solanum, 141
Sorghum, 48
Spectroscope, 85
Sphagnum, 152
Spirogyra, 7, 9, 12
Stamen, 141
Starch, 19, 60, 63, 67, 96, 76-87, 103
Statoliths, 139
Stems, 123
Stigma, 141
Stimulants, 32-33, 79
Stimulus, 16
Stomata, 53-55, 154
Storage, 95-99
Strasburger, 68
Substitutions, 143
Suction, 65-66
Sugar, 13, 19, 35, 71, 86-87, 96-97, 103, 118
Sulphur, 21, 29, 75, 91, 111
Surface, 9, 65
Suspension, 26, 61
Swamp plants, 48
Sweet corn, 97, 104
Sweet peas, 66
Swimming pools, 91

T

Tannin, 21
Tartaric acid, 27
Tarweed, 94, 154
Temperature, 9, 10, 51
Tendrils, 140-141
Tensile strength, 65-66
Tension, 9, 64, 128-131, 141
Thermos bottle, 109
Thermotropism, 152
Tin foil, 83-84, 147
Tomato, 27
Tracheids, 60-71, 96
Tradescantia, 2, 6, 12, 13, 28, 38
Translocation, 70-72
Transpiration, 59, 60, 63
Traube, 43
Tropism, 135-139, 146-150, 152, 157-158
Tropæolum, 52, 55, 57, 149
Tunmann, 21
Turgescence, 3, 4, 12, 129
Turgid, 3, 4, 12
Turgor, 3, 4, 12, 13, 14, 129
Tyndall, 30

U

Ultra microscope, 7
Ultra violet, 148
Urea, 116

V

Vacuole, 22, 37-38
Vascular tissues, 59-72, 128, 134-135
Vaucheria, 87
Vegetable parchment, 40
Vegetative growth, 91

Verbascum, 154
Veronica, 82
Vicia, 123, 136, 153
Vinegar, 27, 120
Viscosity, 7, 9, 25
Vitamin, 74

W

Warm stage, 10
Water culture, 73, 88-91
Waterproofing, 52
Waves, 133
Wax, 53
Weeping, 57
Wheat, 48
Wilting, 13, 14, 68, 70, 129, 151
Wind, 52, 66, 133
Wood, 59-71, 128, 134
Wylie, 144

X

Xanthophyll, 83
Xanthoproteic, 106
Xerophyte, 153
Xylem, 59-71, 128, 134

Y

Yeast, 101, 118, 120

Z

Zinc, 91

265157 Bo
P

Author Peirce, George James

Title Experimental plant physiology.

University of Toronto
Library

DO NOT
REMOVE
THE
CARD
FROM
THIS
POCKET

Acme Library Card Pocket
Under Pat. "Ref. Index File"
Made by LIBRARY BUREAU